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PROGRAMMABLE DNA LATTICES: DESIGN, SYNTHESIS AND APPLICATIONS

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APPROVED: /s/

THOMAS E. RENZ
Project Engineer

FOR THE DIRECTOR: /s/

JAMES A. COLLINS
Deputy Chief, Advanced Computing Division
Information Directorate

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SUMMARY

This contract attacked one of the key challenges facing science and technology in the twenty-first century: namely, the development of programmable methods for construction of complex structured objects on the supramolecular or 10-100 nm scale. Our contract work derived from the recent development (both theoretical and experimental) of self-assembled DNA nanostructures, which provides a methodology for bottom-up nanoscale construction of highly patterned systems. These utilize macromolecular building blocks (“DNA tiles”) based on branched DNA, that self-assemble into periodic and aperiodic lattices. This methodology is *programmable* in the sense that in principle, by the appropriate choice of the set of DNA tiles, the DNA tiling assemblies can be made to form any computable 2Dimensional, 2D, or 3D pattern, however complex, by the appropriate choice of the component DNA of the tiles. In work prior to the contract, we experimentally demonstrated 1D periodic tilings, [YWQ+98, SLM+00], a 1D DNA tiling assembly that can execute computations,[MLR+00], as well as 2D DNA tiling assemblies forming large periodic 2D lattices from a variety of tile motifs, [SLM+00, WWS98, MSS99, LYK00]. The contract work combined the strengths of the leading research groups in DNA lattices: Duke (Reif), NYU (Seeman), and Caltech (Winfree). In the contract work, we extended these experimental techniques in various ways to assemble for the first time: (a) DNA lattices in 2D complex patterning, and (b) periodic 3D DNA lattices. This provided for a highly flexible nanostructure construction methodology: by selectively attaching various other types of molecules to the tiles of the lattices, these lattices can be used as superstructures for placement of nanocomponents composed of a wide variety of other materials. There are many ways this contract impacted Nanoscience. The ability to form programmable patterned nanostructured DNA lattices opens many key opportunities for applied research in nanoscale science and engineering. These include their application as scaffolds and superstructures for aligning proteins and for molecular electronics and nanorobotics. We investigated and experimentally tested major applications of these DNA lattices: (i) periodic 3D DNA lattices were applied to X-ray crystallography of otherwise non-crystalline molecules (which include a considerable percentage of all proteins) that were attached to the lattice, (ii) patterned DNA lattices in 2D and 3D were formed (e.g., we created the patterns for a demultiplexed random access memory, RAM circuit), and (iii) these patterned DNA lattices were used to position molecular electronic components (e.g., molecular wires) and quantum dots into complex patterns. Ultimately, the significance of patterned DNA nanostructures lies not in their existence, but in their application as scaffolds for positioning other materials. Hence it is essential to aggressively further develop these applications. We made a collaborative interdisciplinary research approach spanning many disciplines. We investigated applications in molecular robotics and molecular electronics layout and interconnection, via targeted interdisciplinary research at various other departments at Duke (Chemistry, Pathology, Biomedical Engineering, and Physics). We demonstrated DNA molecular motors composed of DNA and powered by DNA hybridization and enzymes; potential applications of these are to enable and control motions required for assembly of 3D DNA lattices. This contract considerably enhanced technology for nanoassembly, and increased the pace of technology transfer to widespread application to engineering disciplines. Future efforts could build on existing strengths and productive collaborations and provide for the expansion of

DNA nanotechnology by combining with exciting recent advances in peptide, protein, RNA, and carbon nanotube engineering.

1. INTRODUCTION

1.1 Introduction to this research

1.1.1. Main goal of the work.

This was the development of self-assembled DNA nanostructures using bottom-up assembly methods based on self-assembly of DNA molecules into patterned structures, and the application of these DNA nanostructures as superstructures for nanostructures composed of other materials.

DNA is a very promising smart material for constructing periodically patterned structures. DNA also has been designed to direct the assembly of other functional molecules by the use of appropriate attachment chemistries. The diversity of materials which can be chemically attached to DNA considerably enhances the attractiveness of DNA nanostructures for assembly of other materials.

The self-assembly process for bottom-up construction of nanostructures is of key importance to Nanoscience. Self-assembling DNA tiling lattices represent a versatile system for nanoscale construction. The methodology of DNA lattice self-assembly begins with the chemical synthesis of single-stranded DNA molecules, which self-assemble into DNA branched motif complexes, known as tiles. DNA tiles can carry sticky-ends that preferentially match the sticky-ends of other particular DNA tiles, thereby facilitating the further assembly into lattices. Self-assembled two dimensional DNA tiling lattices composed of tens of thousands of tiles have been demonstrated. Self-assembled DNA arrays provide an excellent template for spatially positioning other molecules with increased relative precision and programmability. One potential application of DNA nanotechnology is the use of self-assembled DNA lattices to scaffold assembly of nanoelectronic components, especially metallic nanoparticles. Another is the use of self-assembled DNA lattices to scaffold assembly of proteins and aligning proteins for crystallography studies.

1.1.2. Tangible benefits to end users.

The DNA tile assembly techniques of this contract are the most advanced and versatile system known for programmable construction on the nanoscale. It allows us to build scaffolding on which can be positioned molecular electronics and robotics components. The programmability allows for this scaffolding to have patterning as required for fabrication of complex devices composed of these components.

1.1.3. Critical prior technical barriers.

Prior to this work, we had not yet established routine assembly methods for constructing complex devices out of molecular components. We needed methods to help us hold, shape, and assemble various components into complex machines and systems. The construction of molecular scale structures was therefore one of the key challenges facing science and technology in the twenty-first century. We developed a new theoretical understanding of the nanoscale

world, new software infrastructure, and especially new experimental progress creating molecular structures and machines.

1.1.4. Main elements of the research performed.

This research program was designed to produce a general chemical system to provide control over the structure of matter on the nanometer scale. The ability to do this allowed the design and construction of new materials with defined microscopic features. The research utilized macromolecular building blocks based on branched DNA for the construction of specific nanometer-scale 1D, 2D and 3D periodic lattices. The research results provided new designs for DNA nanostructures, new structural motifs for DNA lattices, novel attachment chemistries enabling nano-patterning of a wide range of materials on DNA lattices, software to assist the design and simulation of DNA nanostructures, and demonstrations of various applications. These results demonstrated the capability of self-assembled DNA nanostructures to provide programmable assembly of DNA molecules into patterned structures, and their application as superstructures for nanostructures composed of other materials.

1.1.5. How our Research overcame the technical barriers.

Prior work had shown that DNA self-assembly can provide arbitrarily complex assemblies, using only a small number of component tiles [Win96]. Prior experiments by the Seeman and Reif groups have demonstrated, for the first time, computation (logical XOR) via molecular assembly of DNA tiles [MLR00]. But prior to this contract, these were not experimentally demonstrated in large scale.

Our experimental results conducted under this contract provided direct evidence of the scalability of our self-assembly approach, and known theoretical results developed under the contract strongly support this. Self-assembled 2D DNA tiling lattices composed of tens of thousands of tiles were demonstrated and visualized by molecular imaging devices such as atomic force microscopes.

1.1.6. Nature of the research results funded under this contract.

The research results provided new designs for DNA nanostructures, new structural motifs for DNA lattices, software and theory to assist the design and simulation of DNA nanostructures, and demonstrations of various applications. These results demonstrated the capability of self-assembled DNA nanostructures to provide programmable assembly of DNA molecules into patterned structures, and their application as superstructures for nanostructures composed of other materials.

1.1.7. Impact of this Funded Research.

This contract dealt with developing systematic procedures for creating complex DNA based patterns in two and three dimensions. In many cases, the formation of these patterns in and of itself can be used to process information. However, the utility of the patterning capacity of DNA can be greatly expanded if it is possible to learn how to translate the complex patterns of base sequences in the DNA into patterns of other materials, with a wider range of physical properties and functions [MLM+96, AJP+96]. As part of this contract we developed the methods for

creating ever more complex nanocrystal patterns, ranging from small groupings of nanoparticles to one, two, and three dimensional assemblies.

Progress in the whole field of nanotechnology would be much slower and more costly without the benefits that DNA nanotechnology can provide. Because most research in nanotechnology appears not to aim at programmable construction and architecture, many of the diverse research projects focusing on materials aspects or molecular electronic devices would have no good integration options once they achieve their results. DNA nanotechnology developed by our efforts provided a programmable scaffolding that will accelerate results in all these areas.

1.2 BREIF REVEIW OF MOTIVATION AND RESEARCH CONDUCTED

The field of nanotechnology holds tremendous promise. There are hopes of achieving vastly better performance for computers and memories, along with a host of other possible applications in materials science, medicine, and biology, if the molecular and supramolecular world could be controlled at will. Due to the promise, numerous research teams have embarked on developing various detailed aspects of nanotechnology, such as strong and electrically active fullerene materials, and organic molecules that have electrical switching properties.

1.2.1 Critical prior technical barriers.

Today the small size of the nanoworld is still more of a liability than a benefit, because we have not yet established routine assembly methods for constructing complex devices out of these tiny components. In the macroscopic world, we have not only human hands, but also mechanical tools of the utmost variety, to help us hold, shape, and assemble various components into complex machines and systems. However, prior to this contract, none of this essential infrastructure existed for the molecular scale. Furthermore, the complexities of the molecular world swamped the limited abilities of our prior theoretical understanding, and new approaches needed to be developed. The construction of molecular scale structures was therefore one of the key challenges facing science and technology in the twenty-first century.

1.2.2 Overview of Other Approaches for Nanoscale Constructs:

(a) Lithography: Conventional top-down assembly methods do not extend to the molecular scale. In particular, lithography, the most successful micro-miniaturization technique so far, will not scale down past the 10nm regime.

(b) Scanning Probe Microscope-Aided Construction: One approach to assembling nanoscale objects is to use an instrument that can move at these size scales, such as a Scanning Probe Microscope (SPM). Serious obstacles to using SPM to construct practical devices include the sequential nature of the technology and its controllability and scalability; although some parallelism exists, it is dwarfed by molecular parallelism.

(c) "Reprogramming" Biological Cells: Biological cells do operate on the nanoscale, but the machinery available in biological cells is exceptionally difficult to predict and control, and we are only beginning to fully understand the complexity of its control systems.

(d) Supramolecular Chemistry and Protein Engineering. This holds great promise for

molecular devices, but it is moving slowly and does not share the programmable nature of biochemistry, such as DNA. Also, this approach when applied to proteins, is at this time limited by the degree of predictability of the resulting protein conformations.

(e) Chemical Self-assembly Methods.

While self-assembly methods are well known and have been long used by chemists, they conventionally result in structures with limited complexity, and are not readily programmable.

1.2.3 Main Goal of the Work.

Our main objective was the development of self-assembled DNA nanostructures using bottom-up assembly methods based on self-assembly of DNA molecules into patterned lattices, and the application of these DNA lattices as scaffolds for nanostructures composed of other materials.

1.2.4 The Methodology of Self-assembled DNA Nanostructures.

This research program was designed to produce a general chemical system to provide control over the structure of matter on the nanometer scale. The ability to do this allows the design and construction of new materials with defined microscopic features. The research utilized macromolecular building blocks based on branched DNA for the construction of specific nanometer-scale 1D, 2D and 3D periodic lattices. For an overview of current work in self-assembled DNA nanostructures, see the recent survey paper: [RLS00]. The methodology of DNA self-assembly begins with the artificial synthesis of single stranded DNA molecules, which self-assemble into DNA branched motifs (tiles), which further self-assemble into large structures with molecular-scale features. In particular, these DNA tiles have sticky ends that preferentially match the sticky ends of particular other DNA tiles, facilitating the further assembly into tiling lattices.

1.2.5 Scalability of the Methodology of Self-assembled DNA Nanostructures.

The experimental evidence provided direct evidence of the scalability of this approach, and known theoretical results strongly support this.

- a) **Theoretical Results:** In principle, DNA self-assembly can provide arbitrarily complex assemblies, using only a small number of component tiles [Win96]. Even with a fixed choice of the component DNA of the tiles, arbitrarily complex tiling assemblies can be formed around input strands of DNA (which are inputs to the assembly program), and general computations can be executed by these tiling assemblies -- which construct the correct output strands during the assembly process [R99, LWR99, WER00, LYK+00]. The error-resilient work conducted in this contract, as described in the following sections provided theoretical evidence of even large scaling.
- b) **Experimental Evidence:** Self-assembled 2D DNA tiling lattices composed of hundreds of thousands of tiles were in prior work demonstrated and visualized by molecular imaging devices such as atomic force microscopes. Recently some novel DNA tiles known as TX tiles have been developed by the Seeman and Reif groups, with properties that facilitate computations [LWR99, LYK+00]. Experiments by the Seeman and Reif groups have demonstrated for the first time computation (logical XOR) via molecular

assembly of these TX tiles [MLR00]. The work conducted in this contract, as described in the following sections provided even larger scaling.

1.2.6 Overview of Tangible Benefits to End Users.

The DNA tile assembly techniques utilized and further developed by this contract were the most advanced and versatile system known for programmable construction on the nanoscale. They allow us to build scaffolding on which molecular electronics and robotics components can be positioned with precision and specificity. The programmability allows for this scaffolding to have patterning as required for fabrication of complex devices composed of these components.

1.2.7 Impact of Research.

Techniques for patterning on the molecular scale were developed via the funding of this research. Progress in nanotechnology was accelerated because most prior research in nanotechnology was not previously aimed at programmable construction and architecture. The research provided many diverse research projects focusing on materials aspects or molecular electronic devices to have a good integration via our methods for building DNA scaffolding. Funding and investment in computational devices including quantum dots would have been less effective without the assembly infrastructure conveyed by this research contract. Likewise, the crystallographic avenues of this contract increased the feasibility of a method based on DNA 3D lattices for capturing proteins will provide determination of the protein structures, which is a much better option than the mercurial nature of the conventional crystallization experiments.

1.3 WORK BREAKDOWN.

Tasks Performed by PI and Specific Contractors:

The various tasks were performed by specific contractors, and their synergistic and collaborative interactions, are given in detail below:

Duke(PI Reif):

We provided overall administration of the contract research.

- We investigated a number of methods for assembly of complex patterns, these include unmediated algorithmic self-assembly (programmed assembly via Wang tiles), step-wise assembly, and our directed nucleation assembly technique (where an input DNA strand is synthesized that encodes the required pattern, and then specified tiles assemble around blocks of this input DNA strand, forming the required 1D or 2D pattern of tiles).
- We also investigated and experimentally tested the use of shape-change induced assembly of 3D DNA nanostructures from 2D DNA lattices (which applies restriction enzymes to targeted sections of a regular self-assembled 2D DNA lattice, and then applies shape changes in DNA lattices induced by either the nano-mechanical transducer devices of Seeman and Yurke or environmental changes). We demonstrated this approach by the assembly of 3D polyhedral objects (e.g. various folded nano structures) from a 2D DNA lattice, by the use of restriction enzymes, and then shape changes in DNA lattices induced by either nano-mechanical devices or environmental changes.
- We designed and characterize various novel DNA tiles, and applied these to generate various DNA tilings. For example, we modified the TX molecules of [LSS99] with

additional Holiday junctions between the top and bottom dsDNA, so the resulting tile, a “Cylindrical TX tile” (CTX), has a cylindrical conformation. We characterized 1 D tilings of CTX tiles which formed long cylinders.

- We developed improved software for design of DNA nanostructures and their DNA sequences. This software included a Java-coded software package for DNA word design for DNA tiles, with a web base interface, *(with Winfree)*. We also developed *(with Winfree)*, software for fast simulation of DNA lattice dynamics using empirical models. In our simulation software, a series of refined models incorporating known empirical results on angular and translational parameters between adjacent b-pair planes, (namely, twist, roll, tilt, shift, slide, and rise) were applied to compute the conformation and dynamics of double helical DNA. The model also was refined to incorporate base sequence dependant variations in these parameters as well as Holliday junctions. Our software applies to single Holliday junctions, to single DNA tiles incorporating multiple Holliday junctions, and to large DNA lattices *(with Winfree)*.
- We also demonstrated methods for creating complex patterns of inorganic nanocrystals via attachment to DNA lattices. (Potentially in the future, in addition to metallic, we can also pattern onto DNA lattices semiconducting, and magnetic nanocrystals.)
- With a collaborator at the Dept. of Pathology (Kenan), we investigated patterned Immobilization of Kinetically-Active Proteins onto DNA lattices. The goal of this subproject was the investigation of hybrid materials composed of self-assembling DNA nanostructures and biological proteins capable of inducible motion for application to nanotechnology. Kenan at the Dept. of Pathology had experience in the construction of libraries of recombinant monoclonal antibody fragments (MAb-equivalents) that can be selected for specificity to virtually any protein. In principal we can re-engineer two different MAbs into a single bivalent "adapter" that can be used to tether any protein of interest to appropriate ribotopes located within a DNA lattice. Initial target structures were included on the DNA lattice as the foundation upon which RNA aptamers were be annealed. To the aptamer we bound an engineered antibody (described below) which also bound to a protein. The whole assembly goes DNA-RNA-antibody- protein. This organized the protein on a self-assembled DNA lattice.

NYU(Seeman): The group at NYU performed the following tasks:

- We designed a variety of DNA motifs expected to form 3D lattices. 2D DNA motifs were used as the basis to organize heterospecies of matter. These included nucleic acid molecules (e.g., knots), nanocrystals, and proteins. DNA motifs appropriate for 2D arrangements were designed as in above.
- We applied current software and software developed by the Winfree and Reif groups to optimize both the geometry and sequences of those DNA motifs.
- We synthesized and purified the strands corresponding to those designs and self-assemble them into the desired motifs. We formed crystalline lattices with the DNA motifs, using the self-assembly methods developed in previous studies.

- When crystals appeared, they were characterized by X-ray diffraction methods. Derivatized crystals were prepared. The structures can be solved when the resolution is high enough to warrant such studies.
- There is only one way to assay the success of 3D periodic assembly: Diffraction. We have produced several preliminary crystalline lattices, and we have obtained diffraction from three of them. In one case, the unit cell dimensions and space group appear to be as designed, although the resolution is not as good as we would like. A key milestone completed was the development of host-lattice crystals that diffract to better than 10 Å resolution. This is not yet a resolution adequate for crystallography, but much better than prior work.
- When resolution of 3D crystals warranted it, one can produce ordered guests within the host lattices. Nucleic acid guests were tethered within lattices, and diffraction experiments performed to establish the extent of their ordering. Nanocrystals were tethered in the same fashion as nucleic acid guests. We needed the high resolution host crystals to assay how well we have tied down the proteins, so experimentation to date was not be highly informative.

Caltech(Winfree): The Caltech team's contribution to this project were in four main areas:

- Mathematical foundations and software technology for design of DNA nanostructures. Building on Seeman's sequence symmetry algorithms, we incorporated additional factors, including more detailed DNA thermodynamics and geometry, into the molecular design models. We also provided algorithms from protein design to reduce the complexity of DNA sequence design. These new algorithms were tested by using them to design the DNA structure
- Several new structural motifs were developed for DNA lattices. We developed small DNA tiles, consisting of few oligonucleotides, which were useful for simplifying experimental procedures. (In the future, we could develop RNA versions of known tile designs, which allow self-assembly to be controlled by *in vitro* transcriptional circuits, thus bridging two important classes of biomolecular computation.) Finally, we modified tile designs for use in step-wise assembly procedures, by adding unique and oriented sites for controlled attachment
- Development of sequential step-wise assembly techniques, and Atomic Force Microscope (AFM) methods for observing and characterizing the assembly. Real-time observation using AFM provided a powerful tool for analyzing and debugging DNA self-assembly procedures. Furthermore, step-wise assembly allowed the parallel synthesis of complex 2D structures using a small number DNA tiles. A theory for step-wise fabrication of 2D and 3D structures was developed. This resulted in what may be a commercially viable general-purpose nanostructure fabrication technique, and we were working closely with our commercial partner, Molecubotics, to develop and commercialize this technique.
- Unmediated algorithmic self-assembly of complex patterns useful as templates for molecular electronics. We studied and surmounted the technical obstacles to unmediated self-assembly by focusing on a test-case, the binary counter, and its extension, the RAM circuit. In combination with the ability to attach molecular electronic components to the

DNA tiles, this provided proof-of-principle for a bottom-up approach to fabricating electronic computers.

A key part of this activity was close collaboration between the four groups. In the initial stage, coworkers from all the groups met a number of times per year, in order to exchange information about the project goals and obstacles. Students and postdocs from the Seeman, and Winfree groups exchanged visits and discussed the research.

2 METHODS, ASSUMPTIONS AND PROCEDURES

The research results provided new designs for DNA nanostructures, new structural motifs for DNA lattices, new chemistries for attachment of various materials to DNA lattices, software to assist the design and simulation of DNA nanostructures, and demonstrations of various applications. These results demonstrated the capability of self-assembled DNA nanostructures to provide programmable assembly of DNA molecules into patterned structures, and the application as superstructures for nanostructures composed of other materials.

2.1 Overview of New Designs for DNA Nanostructures and New Structural Motifs for DNA Lattices (Duke, NYU & Caltech).

A variety of new structural motifs were necessary for other parts of this project, and served as an initial test-bed for the DNA design software. Particular emphasis was on:

- (a) Small DNA tiles, giving us greater resolution in the patterns we make. (The smallest tiles in current use are DAO, at 4 x 12.5nm; we investigated smaller size tiles, e.g., 2 x 6 nm tiles).
- (b) Single-stranded RNA tiles that were generated as run-off transcription products: in mixed RNA/DNA lattices, RNA can be degraded after assembly, leaving only the DNA scaffold in place,
- (c) Tiles generalizing the above mentioned TX tiles, to a more general 3D geometry,
- (d) Large, rigid DNA tiles, investigated for self-assembly.

2.2 Overview of Techniques for Self-assembly of Complex Patterns (Duke & Caltech).

A key portion of the research program was dedicated towards developing the use of DNA to pattern nanocrystals into complex programmable assemblies in one, two and three dimensions.

(a) Unmediated Algorithmic Self-assembly. As mentioned above, unmediated algorithmic self-assembly is Turing-universal [Win96] and so is theoretically capable of creating complex structures from simple rules. Algorithmic self-assembly of complex patterns has useful applications for nanofabrication and arrangement of molecular components. Biological growth

is a proof-of-principle that unmediated biochemical processes can create nanostructures of amazing complexity and sophistication.

(c) Sequential Step-wise Assembly Techniques. Unmediated algorithmic self-assembly of complex patterns required delicate control of physical phenomena such as nucleation rates, crystal growth rates, spontaneous nucleation, and error rates in solutions containing many distinct DNA tile types. An alternative approach also tested was step-wise assembly of a smaller number of DNA tiles under external control via sequential application of different reagents and tiles. We tested this approach for increasing reliability and reuseability during fabrication of DNA nanotechnology structures.

(c) Directed Nucleation Assembly Techniques. We investigated a method for assembly of complex patterns, where an input DNA strand is synthesized that encodes the required pattern, and then specified tiles assemble around blocks of this input DNA strand, forming the required 1D or 2D pattern of tiles.

(d) Shape-Change Induced Assembly of 3D DNA Nanostructures from 2D DNA lattices. We also investigated a method for assembly of 3D DNA nanostructures from 2D DNA lattices. This was done by applying restriction enzymes to targeted sections of a regular self-assembled 2D DNA lattice, and in the resulting fragmented DNA lattice inducing a sequence of shape changes by either the nano-mechanical transducer devices of Seeman and Yurke or environmental changes.

Demonstration of Assembly of a RAM Circuit. Our research in self-assembly of complex patterns focused on a few chosen applications, e.g., we created the pattern for a demultiplexed RAM circuit, which could potentially serve as a template for molecular electronics. The structure of a demultiplexer is nearly identical to the tiling pattern that counts in binary ([RW00]) and attaching molecular electronic logic components to DNA tiles serves as a potential route to arranging the logic components into the desired circuit. We created the demultiplexer RAM pattern using DNA tiles.

2.3 Overview of Applications to DNA-Templated Gold Nanowires and Electronic Components.

Critical for the development of applications of DNA lattices is the development of appropriate attachment chemistry between DNA and the nanoparticle species that one desires to be assembled onto programmable patterns. Networks of nanometer-sized metal or semiconductor islands, or quantum dots, may exhibit a variety of quantum phenomena, with applications in optical devices, nanometer-sized sensors, advanced computer architectures, ultra dense memories, and quantum-information science and technology.

Research here focused on materials fabrication via the programmed assembly of nanoparticles on templates or scaffolds of complex 2D programmable DNA lattices. We demonstrated applications of DNA Lattices to Directed Patterning of Metallic Nanocrystals. Materials displaying useful electronic characteristics were organized into desired patterns of increasing complexity on DNA tile lattices. Collaborators in the Physics department provided expertise in

modeling and measurement of nano-scale electronic behaviors. Synergism was achieved with the Alivisatos group, with whom technical information, materials and working visits were exchanged.

2.4 Overview of Applications of DNA Lattices to 3D Macromolecular Scaffolding (NYU).

DNA lattices were designed to be capable of binding, orienting and juxtaposing a variety of molecules, from receptors and other proteins to organic conductors and optical memory components. The goal was to produce crystalline 3D lattices that can be used to scaffold the subjects of macromolecular X-ray crystallography without the uncertainties of the current crystallization experiment. This development may lead to the ability to examine the 3D structures of biological macromolecules rapidly, eliminating or accelerating markedly the production of crystals, which is currently the rate-limiting step. A spin-off of our results conducted in this contract may in the future be the scaffolding of nanoelectronic systems in 3D [RS87].

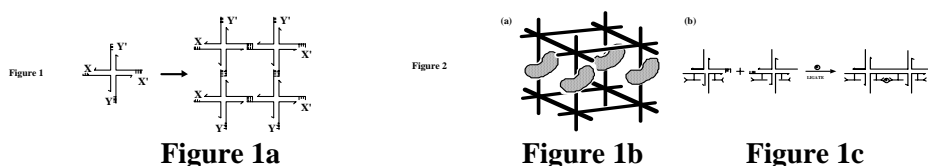


Figure 1: 3D Macromolecular Scaffolding Using DNA Lattices

The system shown in Figure 1, ([S82]), combines stable and robust branched junction motifs with the specificity of DNA cohesive ('sticky') ends. Figure 1a illustrates sticky ends which organize a 4-arm branched junction into a quadrilateral. The sticky ends on the outside would permit this system to be extended to form a 2D periodic lattice. Figure 1b shows how this system can be utilized as a DNA 'box' with sticky ends. Bound to the box are identically oriented biological macromolecules, and the boxes are assembled into 3D host-lattice lattices. Both components may be crystalline, and the entire system may be analyzed by X-ray crystallography. Figure 1c shows that branched DNA offers the promise of being able to scaffold the assembly of nanoelectronic components. Two 4-arm junctions are shown with nano-wire components attached. When the sticky ends on the junctions cohere, the components also come together, so that their assembly is directed by the DNA in a way that would otherwise not occur spontaneously. The system uniquely utilizes both features of DNA cohesion: specific affinity and predictable structure. Specific affinity for DNA associations is used throughout biotechnology and in some nanotechnology (e.g. [MIM+96]), but only this work takes advantage of the fact that the local product structure on both sides of a sticky end can be predicted [QDS97]. *Both of the Figures above utilize motifs simplified to get the concepts across.* The simple 4-arm branches have been supplemented in our work by more robust components [SLM+00, WWS98, MSS99, LYK00] such as double and triple crossover molecules or DNA parallelograms. Our 3D designs have cavities, but are predicated on these other motifs, not the 6-arm junctions.

2.5 Overview of Applications to DNA-Motor Driven Self-Assembly.

We demonstrated *DNA Molecular Motors* composed of DNA and powered by DNA hybridization and enzymes; potential applications of these are to enable and control motions required for assembly of 3D DNA lattices.

2.6 Overview of Software Developed for Assisting the Design and Simulation of DNA Nanostructures (Duke & Caltech).

Software for Design of DNA nanostructures and their DNA sequences (Duke & Caltech).

As DNA computing and DNA nanotechnology become ever more sophisticated, resulting in ever more complex systems, automated design became essential for reliable results. Prior approaches (e.g. [See90]) employ algorithms to minimize the potential for undesired interactions between DNA strands. However, considerable amounts of user input and geometric and chemical insight are still required to produce successful designs. To the extent possible, software was used to automate and/or assist in this process. There are a number of stages to the design of DNA nanotechnology structures: (i) beginning with a preliminary plan of the desired strand topology and geometry; (ii) progressing to a choice of specific lengths of double-helical regions that minimize torsional strain on the molecules; and (iii) choosing particular oligonucleotide sequences that fold into the desired structure, and will not get stuck in alternative or intermediate folds. Currently only this last stage is significantly aided by software [See85]. Although the protein folding and protein design problems are much harder problems than the equivalent nucleic acid folding and design problems, the mathematical framework for the protein problems is more developed because of a larger scientific community. Our project extended the existing mathematical foundation for the DNA design problem, borrowing from experience with protein design when possible, resulting in effective algorithms and software for the design of DNA nanostructures. Although various software tools existed prior to this contract for the design of DNA words used in DNA computations, for certain recombinant DNA experiments, and tiling simulations, these were largely disjoint, and limited in extent of their applications. We provided in this funded research work an integrated system for the design and simulation of DNA nanostructures, which include components for the design of DNA strands used for the DNA tile nanostructures, mechanics simulation of DNA tiles, and kinetics simulations of tiling assembly. (for details, see later sections).

Software for Fast Simulation of DNA lattice Dynamics using Empirical Models (Duke & Caltech).

The software simulation of large DNA lattices containing hundreds of DNA tile nanostructures is an enormous computationally challenging task if we use conventional molecular dynamics simulation software. We circumvented this computational difficulty by developing fast, scalable DNA molecular dynamic simulation software. The software employed an empirical model for DNA dynamic simulation at the base-pair level. DNA chains bend, twist, and stretch in response to base sequence and to specific interactions with the chemical environment. [AM84, Sch85, CL94, HGAS96] extract a DNA model with local energy components, where the coefficients in the model come from empirical results of known DNA X-

ray crystallographic literature. These models were refined to incorporate base sequence dependant variations in these parameters as well as Holliday junctions. The simulation results were reliable enough to provide helpful insight on the dynamics of large DNA lattices. Moreover, the performance of this simulation approach scales linearly with the number of DNA bases of the DNA lattice, so simulations of very large DNA lattices was feasible. A software tool was developed based on this algorithm to allow the user to visualize the dynamics.

3 RESULTS AND DISCUSSION

3.1 New Designs for DNA Nanostructures and New Structural Motifs for DNA Lattices (Duke, NYU & Caltech).

A variety of new structural motifs was necessary for other parts of this project, and also served as a test-bed for the DNA design software. Particular emphasis was put on:

- (a) Small DNA tiles, giving us greater resolution in the patterns we make. (The smallest tiles in current use are DAO, at 4 x 12.5nm; we investigated smaller size tiles, e.g., 2 x 6 nm tiles).
- (b) Single-stranded RNA tiles that can be generated as run-off transcription products: in mixed RNA/DNA lattices, RNA can be degraded after assembly, leaving only the DNA scaffold in place,
- (c) Tiles generalizing the above mentioned TX tiles, to a more general 3D geometry,
- (d) Large, rigid DNA tiles were investigated for step-wise assembly.

3.1.1 Existing structural motifs for DNA lattices.

In prior work we formed 2D lattices from DNA tiles. We did this with three different motifs, double crossover (DX) molecules (Figure 2) of Winfree and Seeman [WLW+98], triple crossover (TX) molecules (Figure 3) of LaBean, Reif and Seeman [LSS99], and Seeman's parallelograms of conventional [MSS99] and modified [SLM+00] Holliday junctions. In addition, we [LSS99] have demonstrated that we can modify the DX tiles (Figure 2), to change the pattern by adding or removing a hairpin, that point out of plane of the lattice [Liu99]. This served as a topographic signal for an AFM, so we could generate predicted patterns that can be visualized with the AFM.

3.1.2 New structural motifs for DNA lattices.

A variety of new structural motifs was necessary for other parts of this project, and also served as an initial test-bed for the DNA design software:

- (a) Small DNA tiles.** The smallest tiles currently in use are DAO, consisting of 4 oligonucleotides arranged in a 4 x 12.5nm unit. Smaller tiles, requiring fewer strands, gave us

greater resolution in the patterns we make, improve stoichiometry, reduce costs, and facilitate experimental procedures. A possible tile design is shown in Figure 4. This design consists of two oligonucleotides per tile, and has dimensions of roughly 2 x 6 nm. Care must be taken to ensure that self-assembly produces flat two-dimensional lattices; a good test of our design methods.

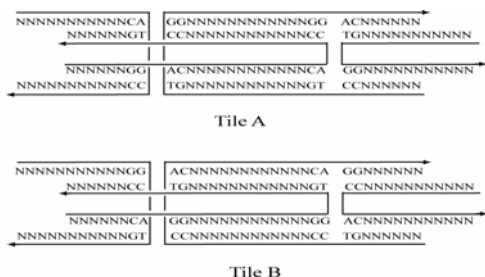


Figure 2A: A DX Tile



Figure 2B: A DX Lattice

(b) Cylindrical TX tiles (CTX tiles). It is possible to modify the triple crossover (TX) molecules (Figure 3) of LaBean, Reif and Seeman [LSS99] and their extensions, with additional Holiday junctions between the top and bottom dsDNA, so the resulting tile has a cylindrical conformation. These may form tilings that have potentially interesting properties, e.g., 1D tiling of CTX tiles may be used to form long cylinders and may be able to capture linear structures (e.g., carbon nanotubes). A number of interesting 2D and 3D tilings based on hybrids of CTX plus more conventional DX and TX tiles are also possible. As a start in this direction, the Seeman laboratory has recently produced 6-helix bundle DNA nanotubes whose lengths are routinely in the 1-10 micron range [MMS01].

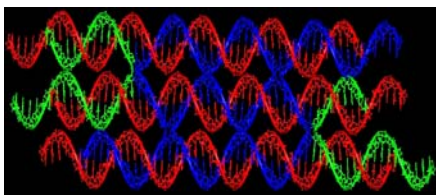


Figure 3A: TX tiles

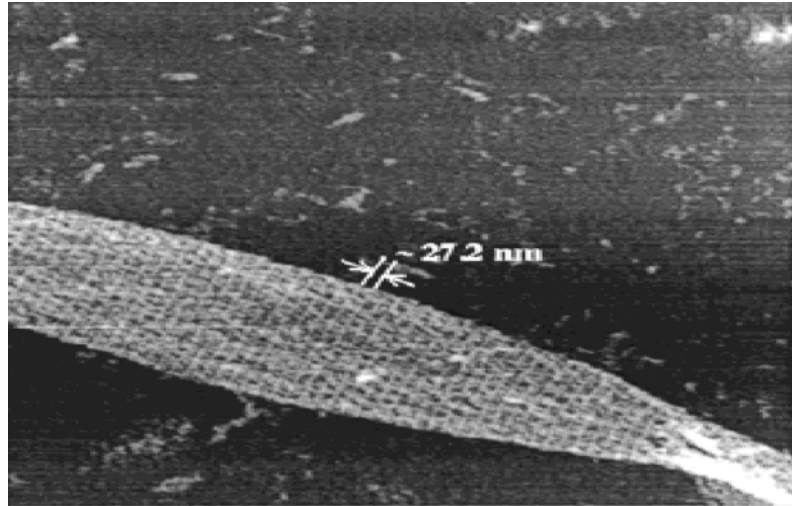


Figure 3B: A TX lattice

(c) RNA tiles. Tile designs using RNA instead of DNA could in the future open up several new possibilities. In mixed RNA/DNA lattices, RNA can be degraded after assembly, leaving only the DNA scaffold in place. This theoretically increases the complexity of structures that can be assembled [RW00]. Additionally, RNA tiles that can be generated as run-off transcription products allows real-time control of self-assembly processes, including staged and feedback control by *in vitro* transcriptional circuits that determine *when* the tile is produced. The techniques of staged and feedback control of self-assembly processes is commonly used in biological cells for the fabrication of complex devices; for example, the self-assembly of the flagellar motor and flagellum in bacteria.

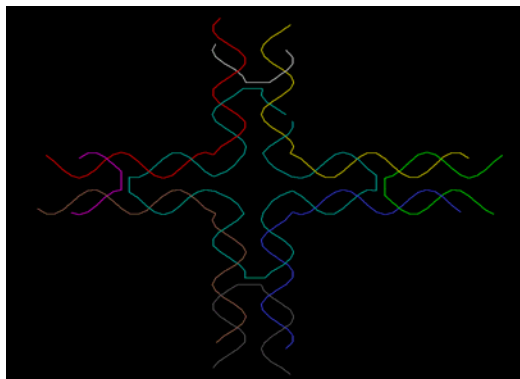


Figure 4A: A 4 x 4 Tile

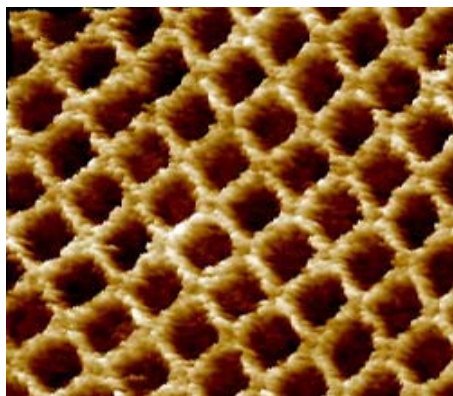


Figure 4B: A 4 x 4 Lattice

In work funded by this contract, Duke succeeded in design, assembly, and characterization of a new type of DNA tile (referred to as 4x4) and lattice formed from 4x4 tiles (Figure 4). Improvements over previous tile structures include a square aspect ratio which will help regularize the pixel array, sticky-end connections in four directions (north, south, east, and west) within the lattice plane rather than the two direction (east and west) connections utilized in most previous DNA tile arrays (DX and TX tiles). Self-assembly of hydrogen-bonded two-dimensional array with this motif generate ‘waffle’ like lattices with repeating cavities of ~15.6x15.6 nm. We targeted gold nanoparticles to the 4x4 DNA lattices and plan to execute conductivity measurements on the metallized 4x4 DNA lattices. We also targeted proteins and other macromolecules to the cavity produced by self-assembly of this lattice. We designed and built a variant 4x4 lattice in which neighboring tiles are flipped bottom-to-top through the lattice plane so tiles alternate their orientation such that any deviations in the tile structure from the ideal will tend to cancel each other out instead of being accumulated. This “corrugated 4x4” structure is able to form larger lattice sheets and fewer undesired, long tubular structures. The Duke group has also produced further variants of the new 4x4 DNA tile. One 4x4 variant contains strands which run through the tile from top-to-bottom and right-to-left which can be used as scaffold strands. The scaffold strands act as nucleation lines for a 4x4 lattice and can be used to program surface patterns on 2D lattices. This results on a TXx4 tile that is composed of 4 TX tiles joined around a central cavity similar to the 4 four-arm junctions in the 4x4 structure. We also demonstrated the immobilization of streptavidin onto well-formed a 4x4 lattice via binding to biotin molecules covalently bond to oligonucleotides integral to the 4x4 tiles.

3.1.3 Experimental Construction of DNA nanotubes (Duke & Caltech)

In work funded by this contract, Duke and Caltech independently each formed long cylinders of DNA tiles which morphologically similar to carbon nanotubes). Duke investigated modifications of the TX molecules with additional Holiday junctions between the top and bottom dsDNA, so the resulting tile, a “Cylindrical TX tile” (CTX), has a cylindrical conformation. We demonstrated the use of these and related families of DNA tiles to form 3D DNA lattices based on hybrids of CTX plus more conventional DX and TX tiles. We also formed DNA tubes from 4 x 4 tiles, (Figure 5).

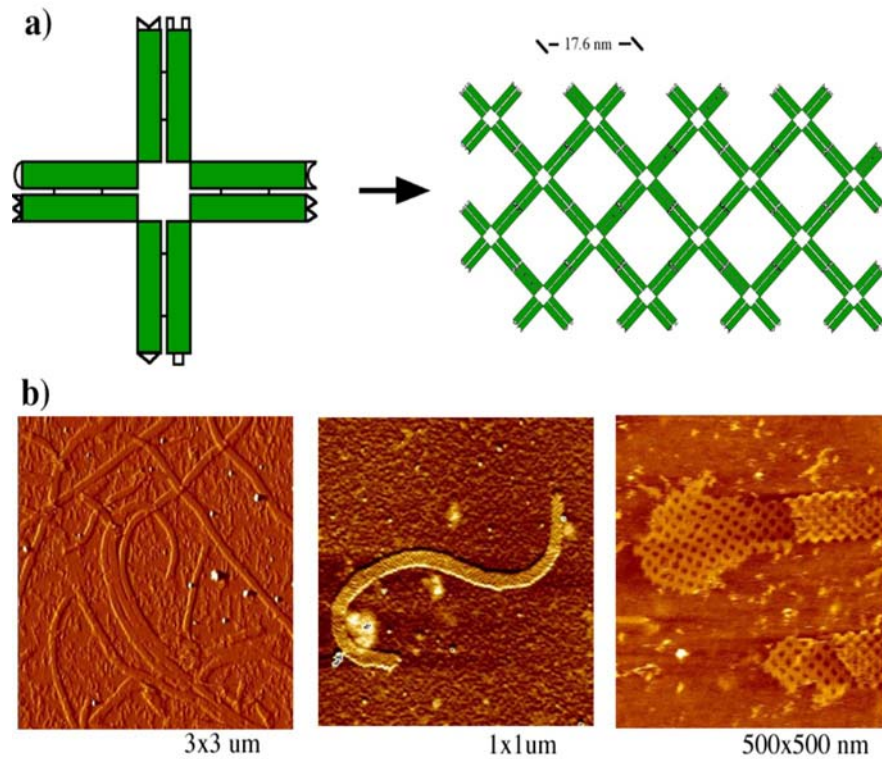


Figure 5: DNA tubes assembled from uncorrected 4 x 4 tiles.

Similar to the Duke efforts described above, Caltech researchers have encountered tubular structures formed by DNA tiles, in this case TX and DAE double-crossover tiles with no chemical modifications. AFM microscopy indicates that the DAE tubes consist of between 4 to 8 tiles in cross-section, with the long axis of the tube equal to the long axis of the tiles. We are collaborating with Deborah Fygenson at UCSB to study the biophysical characteristics of these tubes, such as the persistence length (tensile strength) and the kinetics of assembly, and the design determinants for lattice formation [A. Nkodo, P.W.K. Rothmund, N. Papadakis, E. Winfree, D. Fygenson, “Programmable Self-assembly and Dynamics of DNA Nanotubes”].

3.1.4 Experimental Demonstration of very high resolution (~2nm) imaging of DNA structures under physiological buffers and in real time (Caltech)

Caltech made an investigation of various assembly techniques for patterned 1D and 2D DNA lattices of moderate length, using techniques of unmediated algorithmic self-assembly and directed nucleation assembly. Caltech achieved very high resolution (~2nm) imaging of DNA structures under physiological buffers, which is giving us greater insight into the geometry of the lattices. Additionally, we have been able observe crystal formation in real time, which will allow us to study and control 2D crystal growth on the mica surface. We continued to develop AFM procedures for continuous imaging of self-assembled DNA structures, thus permitting us to make movies of the growth of tile-based DNA assemblies. These movies promise to provide insights

into growth and error rates – a sort of “visual debugger” for self-assembly programs. Key issues investigated included characterizing how growth on the surface differs from growth in solution, how to ensure uniformity of the surface, and how growth and uniformity can be controlled by changing concentrations, temperature, and buffer conditions.

3.2 Techniques for Self-assembly of Complex Patterns (Duke & Caltech).

A key portion of our research program was dedicated towards developing the use of DNA to pattern nanocrystals into complex programmable assemblies in one, two and three dimensions.

3.2.0 A Test Challenge: Assembly of a RAM Circuit.

In addition to being a unique intellectual challenge that yields fundamental insight into biomolecular computation, DNA self-assembly has useful applications for nanofabrication and arrangement of molecular components. For the contract, we focused on a particular application: to create the pattern for a demultiplexed RAM circuit, which could potentially serve as a template for molecular electronics.

A RAM circuit essentially consists of a periodic lattice of memory elements coupled to a means to specifically address any given element for writing or reading a logical bit. A RAM containing 2^N memory elements requires N address bits. The circuit that uses these N input lines to specify a single element is known as a demultiplexer. Interestingly, the structure of a demultiplexer is nearly identical to the tiling pattern that counts in binary [RW00]. Thus, attaching molecular electronic logic components to DNA tiles serves as a potential route to arranging the logic components into the desired circuit, (Figure 6).

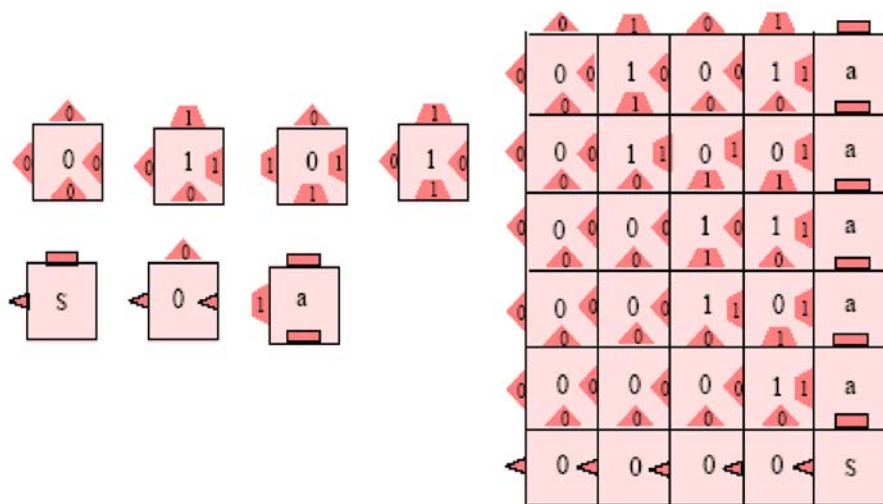


Figure 6: Tiling Assembly of Binary Counter (Winfree)

The Winfree group has achieved success with this DNA self-assembly demonstration. Since molecular electronics are available to implement the memory and logical cells, and can be attached to the DNA tiles, a functional RAM circuit can in the future be fabricated with unit cells

on the order of 10nm or less. In the future, it would be of great interest to explore, theoretically and experimentally, what other common circuit layouts can be achieved by algorithmic DNA self-assembly.

3.2.1 Unmediated Algorithmic Self-assembly (Reif & Winfree).

The most general method we investigated for 2D pattern formation is the use of a small set of DNA tiles that self-assemble in a predictable manner. As mentioned above, unmediated algorithmic self-assembly is Turing-universal [Win96] and so is theoretically capable of creating complex structures from simple rules. This method has the advantage of being very general; it required no input DNA strand encoding a pattern, and instead the 2D pattern is essentially generated by the choice of the DNA tile set. The class of patterns generated by such a “Wang Tiling” has been shown to include, in principle, all computations. (For an overview of this method of pattern formation, and a number of examples, see [RLS00] and its referenced URL as well as [Win96, WYS98, Rei99, LWR00, LYK+00].) Algorithmic self-assembly of complex patterns has useful applications for nanofabrication and arrangement of molecular components. Unmediated algorithmic self-assembly of complex patterns required delicate control of physical phenomena such as nucleation rates, crystal growth rates, spontaneous nucleation, and error rates in solutions containing many distinct DNA tile types.

An example of an algorithmic self-assembly process is given in Figure 6, which shows a set of 7 tiles that self-assemble from the “seed tile” **S** and thereby count in binary; each subsequent row, from bottom to top, encodes an incremented binary number. Logically, a unique, correct pattern is produced if a new tile is added only when at least two sides match perfectly. However, the self-assembly of DNA tiles, implemented by DX, TX or other structures, is intrinsically stochastic. Theory and computer simulations based on chemical rate equations show that near the melting temperature of the 2D DNA lattice, low error rates can be achieved [Win98]. In this contract, this was refined and demonstrated experimentally; in particular, two issues were investigated: error rates for tile addition, and proper nucleation from the seed tile. Low error rates had already been observed in the first algorithmic self-assembly performed [MLR00]; however, the physical determinants of these error rates need more thorough study.

We studied these phenomena using a DNA implementation of the binary counter tiles as a prototypical 2D DNA self-assembly, and compare algorithmic self-assembly with the two above alternative methods: sequential step-wise assembly and directed nucleation assembly.

The first experimental demonstration of aperiodic patterned 2D DNA lattice using Boolean computations (Caltech)

In work begun when initially funded by this contract, Caltech succeeded in experimentally demonstrating their novel method to construct an aperiodic patterned 2D DNA lattice (Sierpinsky Triangle Lattices) by a self-assembly process utilizing Boolean computations by DNA tiles. Caltech began by identifying strategies for patterning surfaces at the nanometer scale, including patterns required for nanoelectronic circuits, such as a RAM memory array and addressing circuits. We then moved this project toward experimental realization using algorithmic DNA self-assembly, beginning with the goal of an unbounded binary counter (a component of the demultiplexing RAM pattern). Caltech designed and synthesized strands for a

two-dimensional algorithmic self-assembly system, and has shown by atomic force microscopy that both 1-tile-thick and 2-tile-thick linear borders for the lattice form reliably. We used two approaches: (i) We doped this assembly with a seed to grow an X-shaped border. The 2x2 nucleus then forms and was imaged. Experiments with extended X-shaped structures have indicated that greater theoretical understanding is necessary. We continue this investigation using our real-time movie technology to watch growth of the patterns from defined seeds. (ii) Sidestepping these issues, Caltec successfully demonstrated algorithmic self-assembly from the alternative method of using long PCR-generated template strands. In the best samples, error rates are between 1 and 3 percent per step, and perfect assemblies with over one hundred tiles have been observed.

3.2.2 Directed Nucleation Assembly Techniques (Duke).

We investigated another method for assembly of complex patterns, where an input DNA strand is synthesized that encodes the required pattern, and then specified tiles assemble around blocks of this input DNA strand, forming the required 1D or 2D pattern of tiles.

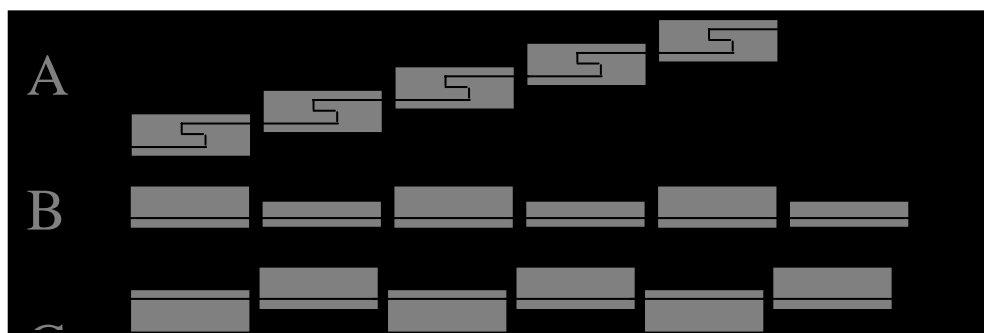


Figure 7: Molecular Pattern Formation using Scaffold Strands for Directed Nucleation:

Multiple tiles of an input layer can be assembled around a single, long DNA strand we refer to as a scaffold strand (shown as black lines in the figures). This method makes the use of artificially synthesized DNA strands that specify the pattern and around which 2D DNA tiles assemble into the specified pattern; in this method, the permanent features of the 2D pattern are generated uniquely for each case (see Figure 7).

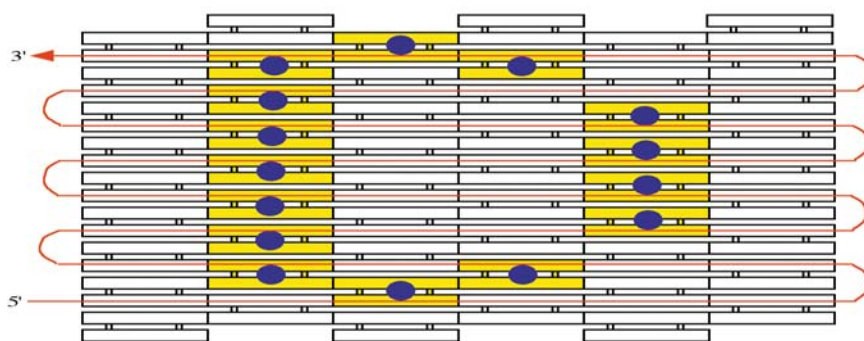


Figure 8: Example of Duke's Directed Nucleation Assembly Technique for Generating 2D patterning.

In Figure 8, red is an input ssDNA “pattern” strand. It encodes a 2D pattern in modified row major order (each odd row traversing alternately left to right, and each even row traversing right to left). Specific DNA tiles self-assemble around each segment of this input “pattern” strand. The tiles then self-assemble into a 2D tiling lattice with the predetermined pattern defined by the “pattern” strand. In prior work, a small instance of this method has been successfully executed where up to 10 TX tiles assembled around a preformed scaffold strand [LWR99] on a linear 1 D DNA array.

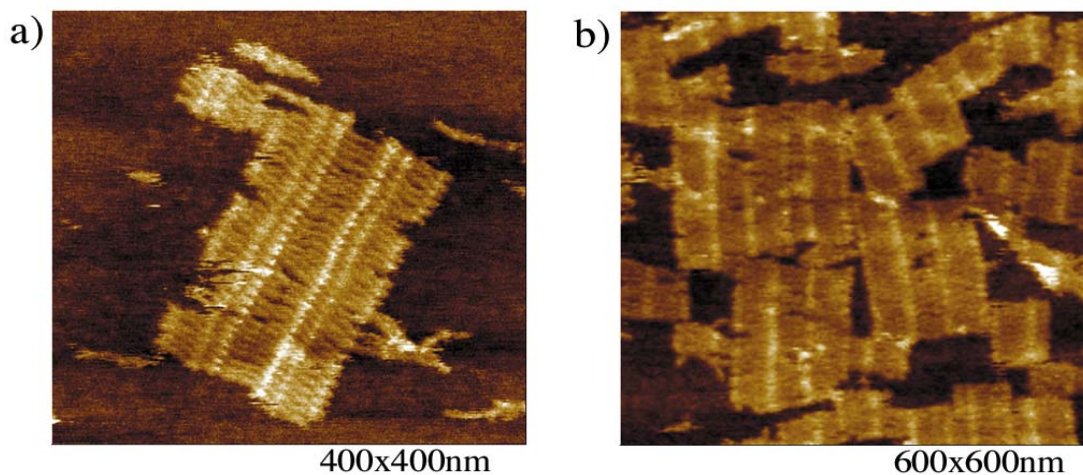


Figure 9: Experimental Demonstration of a Barcode Patterning via a DNA Lattice

We experimentally demonstrated the first aperiodic patterned DNA lattice (Barcode Lattice) by a self-assembly process utilizing directed nucleation of DNA tiles around a scaffold DNA strand (Duke). This demonstrated the transfer of information from a single strand of DNA into an observable pattern on large lattice sheets (1D \rightarrow 2D information transfer). The displayed patterns were designed to be more complex than the simple periodic patterns so far demonstrated in DX

and TX tile superstructures. Duke succeeded in constructing “barcode” DNA tile lattices which display microscopically observable banding patterns, (Figure 9). As a first step, the patterns are instantiated with a single pixel per tile and two possible states per pixel determined by the presence or absence of an extra stem-loop of DNA on the tile face projecting out of the lattice plane. Results were obtained by examining assembled structures using atomic force microscopy (AFM). The formation and observation of this barcode lattice is equivalent to the transfer of information encoded on a 1-dimensional strand of DNA onto a 2-dimensional DNA lattice. Therefore, this technique may be adapted to function as a visual readout method.

We succeeded in producing a patterned lattice assembled around scaffold strands spanning five tiles (~60 X 70 nm) and by adding sticky ends to the flanking tiles we were able to produce a large (~3 X 10 micron) lattice which displays the target banding pattern with 5-tile periodicity. This work is the first directed nucleation assembly of barcode patterned DNA Lattices. We also made an implementation of a second 5-tile barcode pattern. The patterning is determined by a 327-base scaffold DNA strand constructed by ligation of shorter synthetic DNA oligos. The scaffold DNA strand provides to the DNA lattice barcode patterning information represented by the presence or absence of DNA hairpin loops protruding from the DNA lattice. Self-assembly of multiple DNA tiles (in this case 5 tiles) around the scaffold strand was demonstrated to result in a patterned lattice containing barcode information of 01101.

Further demonstrations of DX barcode lattices were completed in order to show the ease with which the surface patterns can be reprogrammed. We have constructed and observed aperiodic tile lattices displaying another pattern, 10010. The information for the patterns was encoded on a scaffold strand of DNA as stem-loops sticking out of the tile plane and was observable by AFM. This result advances our efforts both to work toward displaying more and more complex surfaces patterns and also to develop a visual output method which translates 1D information encoded on a DNA strand into 2D pictures displayed on a DNA lattice surface.

We obtained AFM images of pieces of barcode lattice formed by directed nucleation of DX tiles; the 600x600 nm AFM image of barcode lattice designed to display an aperiodic pattern of stripes. SPM imaging has been essential for evaluation of these structures. A recent paper, which experimentally demonstrated for the first time our method for programmed molecular patterning, was published in [YLF+03].

We also developed further increases in the length of the repeat unit, as well as construction of general tile sets for propagating 1D information into observable 2D banding patterns. We designed a 2D programmable barcode tiling system which reuses the scaffold strand on each layer. Layers can now be annealed individually and then combined sequentially in order to add to the growing lattice.

3.2.3 Sequential Step-wise Assembly Techniques (Duke & Caltech).

An alternative approach (first described in [R99]) is step-wise assembly of a smaller number of Molecular Building Blocks (MBBs) under external control via sequential application of different reagents and MBBs. We tested this approach for increasing reliability and reusability during fabrication of DNA nanotechnology structures.

3.2.4 A scheme for 2D patterning on a 2D DNA lattice (NYU)

NYU developed a theoretical scheme for 2D patterning on a 2D DNA lattice using an addressable pegboard scheme.

3.2.6 Schemes for patterning of 2D DNA lattices of Counters and MUX Circuits (Caltech)

The self-assembly of DNA tile nanostructures into 2D and 3D lattices can be used to manufacture patterned nanostructures from smaller unit components known as DNA tiles. Figure 10 gives an example of a tiling assembly whose rows provide a sequence of numbers that count upwards.

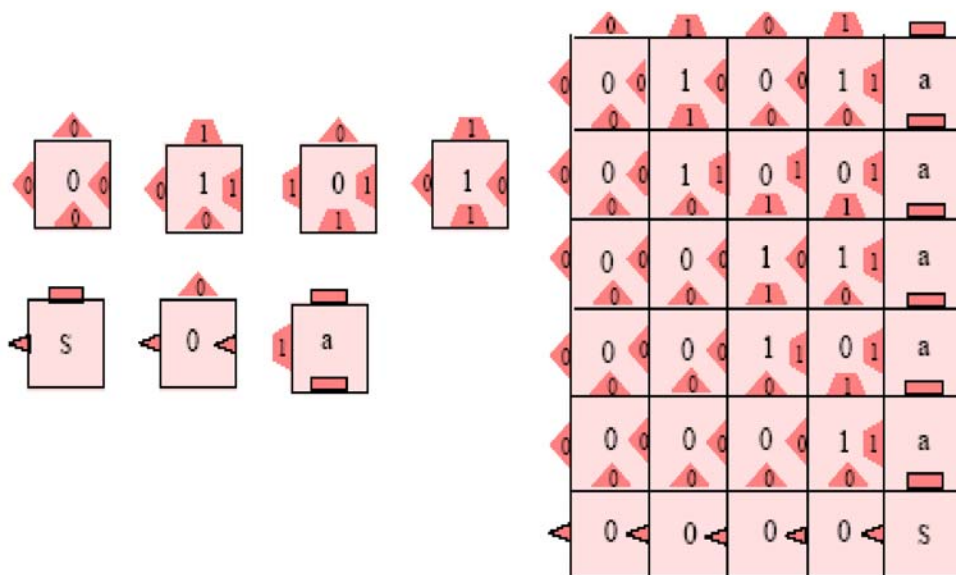


Figure 10: Example of a Computational Error

A somewhat more complex assembly can be used to form demultiplexed random access memory, commonly used in RAM circuits for transforming binary addresses to unary addresses.

3.2.7 Error Correction Methods for Self-Assembly (Duke & Caltech)

The assembly of Figure 10 provides an example of a tiling lattice where a single error will make the rest of the assembly incorrect. However, self-assemblies at the molecular scale are prone to a quite high rate of error, and the key barrier to large-scale experimental implementation of DNA tiling is the high error rate in the self-assembly process. One major challenge to nanostructure self-assembly is to eliminate/limit these errors.

In work begun when initially funded by this contract, Winfree provided an innovative approach to decrease tiling self-assembly errors without decreasing the intrinsic error rate of assembling a single tile. However, his technique resulted in a final structure that is four times the size of the original one.

In a recent paper Duke [RSY05] developed a compact error-resilient tiling method that does not increase the size of the tiling assembly. This method applies to assembly of Boolean arrays which perform input sensitive computations (among other computations). Our error-resilient tiling uses multi-way overlay redundancy such that a single pad mismatch between a tile and its immediate neighbor forces multiple further pad mismatch between a pair of adjacent tiles in the neighborhood of this tile. This drops the error rate substantially. Theoretical stochastic analysis and empirical studies of the computer simulation of Sierpinsky Triangle tilings have been used to validate these error-resilient tiling results, and indicated that the speed of the assembly was not reduced.

3.3 Application DNA Lattices to Directed Patterning of NanoElectronic Particles and Proteins

3.3.1 Metallic Nanocrystals and quantum dots and wires (LaBean & Reif with Gleb Finkelstein, Dept. of Physics, Duke University.)

We used self-assembling DNA templates to fabricate nanostructures with novel and technologically significant electronic transport properties. A specific objective was the demonstration of a molecular quantum dots using the DNA metallic templating technique. We have incorporated thiolated oligonucleotide into DNA lattice such that -SH groups are displayed on the ends of helical stems protruding from the lattice at fixed sites. Au nanoparticles are then bonded to the immobile sulfurs. Alternatively, thiolated oligonucleotide is reacted directly with Au to yield single-strand DNA labeled Au which is subsequently annealed to its complementary strand displayed on the lattice on protruding stems. As shown in Figure 11 and Figure 12, the final step in the production of long continuous wires involves fusion of the immobilized spheres in the presence of gold salt and hydroxylamine [BN98].

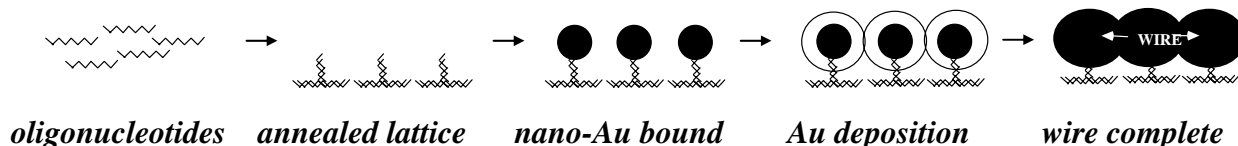


Figure 11: Metallization of a DNA Lattice

We used DNA templates to fabricate chains of Au nanospheres that range from non-touching, in which case tunneling is the primary conduction mechanism, to continuous wires with periodically modulated cross section. Among high-field phenomena we examined are hysteresis in current-voltage characteristics as single charges jump on and off isolated Au nanospheres and negative differential resistance effects that may occur in the continuous wire system due to one-dimensional mini-band formation. We also studied the RF conduction properties of our structures which provided critical information about the ultimate speed of operation of nanostructure-based devices [DS00]. In order to interpret data on electronic transport through the fabricated structures we studied phenomenological models of charge transport in periodically modulated nanoscale metallic wires.

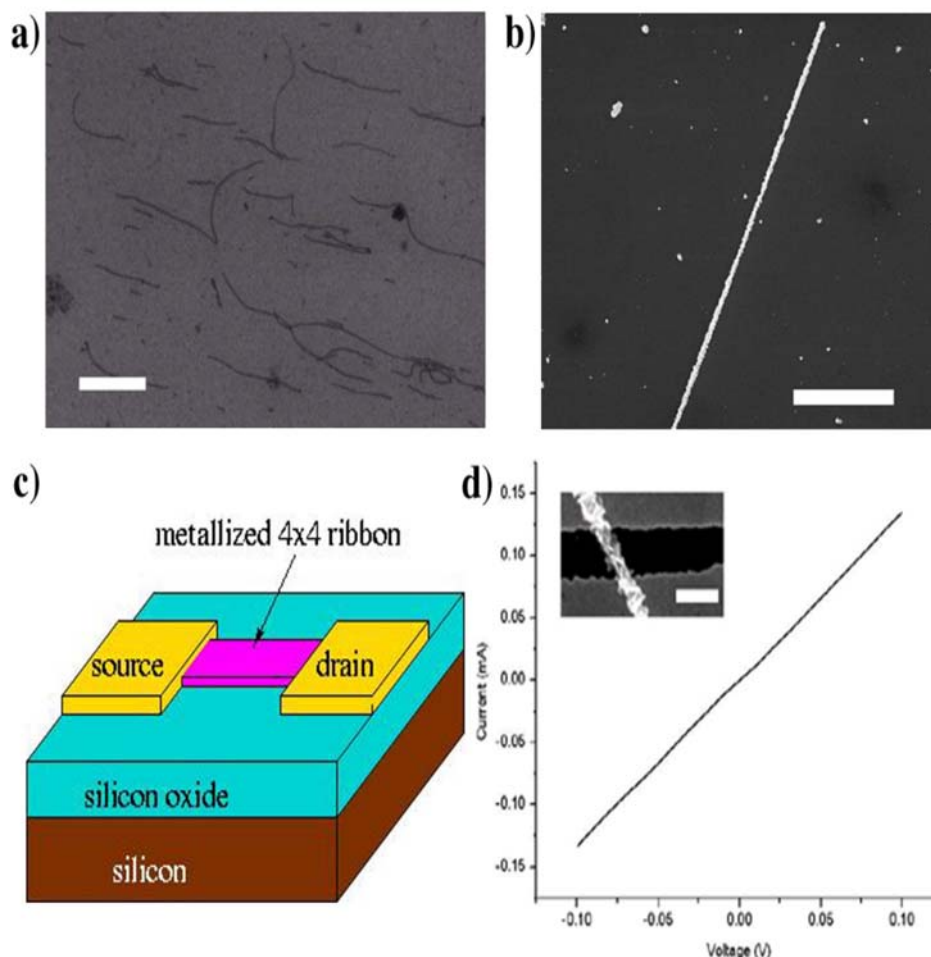


Figure 12: Au Metallization of 4x4 DNA Tube and Conductivity Measurement

Duke made significant advances toward nanoelectronic goals including successful metallization of TAO lattice nanotubes with specific nanogold attachment and targeted silver deposition on silicon oxide substrate. Electron beam lithography techniques are currently being used to write electrical contacts to the metallized DNA nanotube wires. We have succeeded in both halves of the process, construction of fully metallized DNA nanotubes and e-beam electrode writing. We are now combined the two. Current/voltage measurements on single wires were completed and demonstrated conductivity. Comparison were be made between wires deposited onto preformed electrodes and wires deposited first followed by specific electrode etching.

Duke also investigated alternative DNA metallization strategies including the glutaraldehyde/silver metallization and the use of gold enhancement (NanoProbes, Inc.) since gold is more resistant to oxidation than silver.

3.3.2 The experimental Demonstration of the assembly of proteins onto targeted locations on DNA lattices.

In work funded by this contract, Duke demonstrated they were able to use self-assembled DNA nanostructures to precisely control the spatial location of both streptavidin molecules and their nanogold conjugates. In particular, [PYR2004] describes the use of linear TX arrays for the assembly of streptavidin conjugated 5-nm gold particles, where the gold is precisely positioned periodically on the self-assembled DNA array. The specific biotin streptavidin interaction, combined with the programmability of DNA nanostructures, leads to more complex patterned structures with addressable features, (Figure 13). The organization of streptavidin conjugated gold nanoparticles into periodic arrays templated by DNA nanostructures provides a convenient way to construct multiple nanoparticle arrays for electrical measurements by increasing the size of the nanogold. It may also find applications in constructing logical molecular electronic devices such as quantum cellular automata or serve as interconnects between other nanoelectronic and molecular electronic components by providing uniformly sized gaps between adjacent gold nanoparticles.

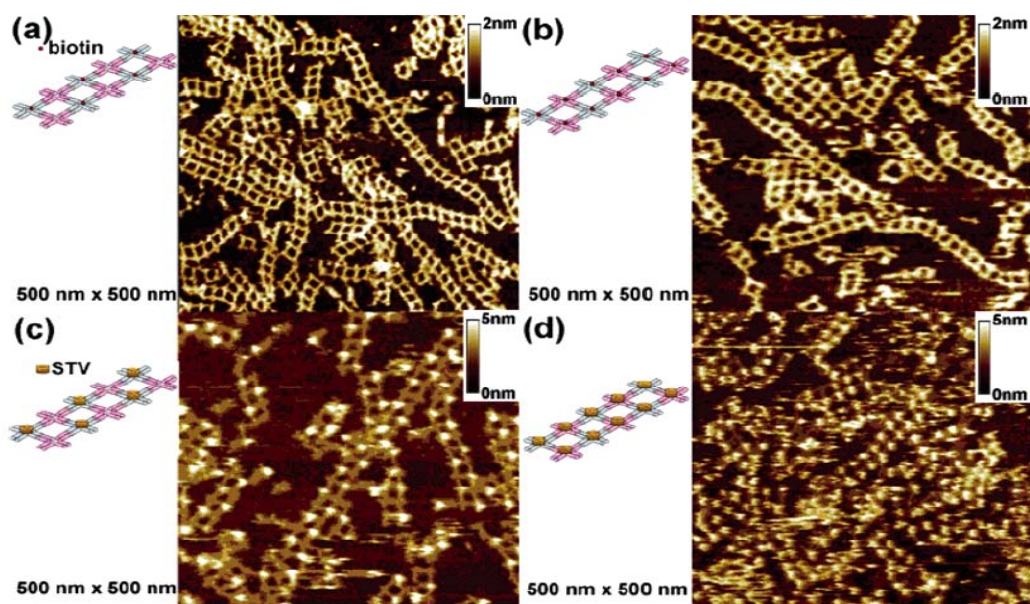


Figure 13: Linear DNA TX lattices with biotin covalently bound to DNA tiles

Duke targeted gold nanospheres into desired patterns by templating with DNA lattices. Our long-term goal has been to use self-assembling DNA templates to fabricate nanostructures with novel and technologically significant electronic transport properties. Materials displaying useful electrical characteristics are organized into desired patterns via the self-ordering properties of DNA complementarity. We made use of well-known gold-sulfur chemistry for binding of gold nanospheres to our DNA lattice. First, thiolated oligonucleotide was incorporated into the DNA

lattice such that the -SH group is displayed on the free end of the stem helix protruding from the lattice at fixed sites. Gold nanoparticles have been added to the annealed lattice and bind the immobile sulfur. Alternatively, thiolated oligonucleotide can be reacted directly with gold nanoparticles to yield single-strand DNA labeled gold which can be subsequently annealed to its complementary strand displayed on the lattice on protruding stem helices. We employ a DNA lattice to impose patterns of interest on the assembling gold, thereby allowing increasingly complex constructs with only small changes in the overall scheme. The final step in the production of long continuous wires involves fusion of the immobilized spheres in the presence of dissolved gold salt and hydroxylamine. We incorporated thiol containing oligonucleotides into a TAO AB lattice with surprising results - the thiols formed disulfide bridges under the conditions tested which distorted the flat lattice sheets into regular sized filaments. We also demonstrated metallization and conductivity of the filament structures.

Duke has also demonstrated another method of patterning nanoparticulate gold on a DNA lattice. 5 nm gold nanospheres were each specifically labeled with a single copy of the central oligonucleotide of the 4x4 tile. Annealing of this labeled oligo with the remainder of the strand set resulted in organization of 5 nm gold particles in the expected pattern.

3.4 Application of DNA Lattices as Macromolecular Scaffolding (NYU).

3.4.1 Introduction to Macromolecular Scaffolding.

This research program was designed to produce a general chemical system to provide control over the structure of matter on the nanometer scale. The ability to do this allows the design and construction of new materials with defined microscopic features. The utilized macromolecular building blocks based on branched DNA for the construction of specific nanometer-scale 1D, 2D and 3D periodic lattices. The primary emphasis were on 3D periodic systems, because we had already succeeded in producing a number of 1D [YWQ+98, SLM+00] and 2D [WWS98, MSS99, LYK+00] systems. The goal of developing the system was to provide a macromolecular scaffolding, capable of binding, orienting and juxtaposing a variety of molecules, from receptors and other proteins to organic conductors and optical memory components. The key specific goals were to produce crystalline 3D lattices that can be used to scaffold the subjects of macromolecular X-ray crystallography without the uncertainties of the current crystallization experiment. This effort led to the ability to examine the 3D structures of biological macromolecules rapidly, eliminating or accelerating markedly the production of crystals, which is currently the rate-limiting step. A likely spin-off of this effort is the scaffolding of nanoelectronic systems in 3D [RS87].

The basic idea behind this system is to combine stable and robust branched junction motifs with the specificity of DNA cohesive ('sticky') ends. This concept was first enunciated by Seeman in the early 1980's [See82]. Assembly is directed by the DNA in a way that would otherwise not occur naturally. Thus, once we have produced host-lattices, not only are we able in principle to orient biological macromolecules, but we were able in principle to organize nanoelectronic components.

3.4.2 3D Host Lattices.

The Seeman lab had been working for many years to generate 3D crystals of DNA lattices. He has produced several arrangements that have managed to crystallize. The first of these is a 3D extension of a 2D lattice reported previously based on DNA triple crossover (TX) molecules [LYK+00], which is illustrated in Figures 14a and 14b.

Shown on Figure 14a is a schematic of the 2D lattice, and an AFM of it is on Figure 14b. Triple crossover molecules contain three antiparallel helices held together by strand crossovers, and are roughly planar in this case. The AB lattice shown is connected 1-3, as indicated by the geometric complementarity of the ends of the helices; this arrangement produces a gap that can be filled by a third TX (C) that has been rotated 3 nucleotide pairs (103°) to become C'. C' is held into the arrangement by its middle helix, so that the outer helices protrude from the planar arrangement; these protruding helices produce the striped features seen on the right of Figure 14b. The D component is just a helix here, but if it is replaced by a component similar to C', the system extends to 3D, as suggested by Figure 14c:

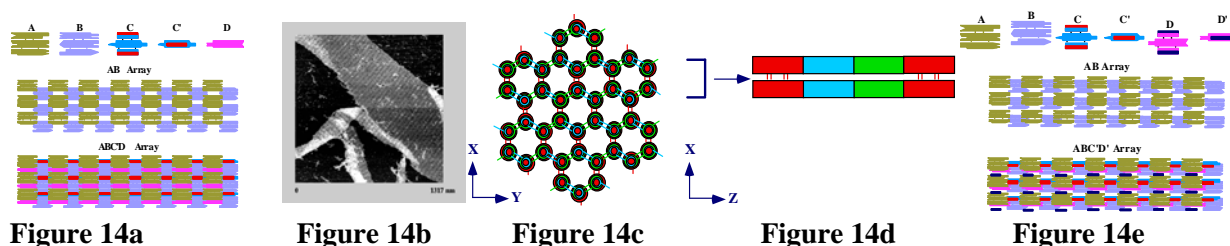


Figure 14: The design of 3D DNA Lattices from 2D DNA Lattices

The idea is that the sticky ends on the top helix of C' complement those on the bottom helix of D' and vice versa. The sticky ends that extend out of the plane have been removed for simplicity. Crystals have been produced from this motif, and we await diffraction from them. A design that leads to a simpler asymmetric unit (~ 140 nucleotides, rather than ~ 1240) is based on a double crossover (DX) motif. This arrangement is shown in Figure 14d and 14e. The system is a trigonal crystal, designed to be space group $P3_1$. The idea is that 10.5-fold DNA means that 14 nucleotide pairs correspond to about $4/3$ turns of DNA. Each double crossover molecule is separated by 14 nucleotide pairs from the one below it, thereby rotating it 120° . This system ramifies into a three-fold screw axis. The schematic in Figure 14c shows a top view of the structure. Each circle represents $4/3$ of a helical segment, with red both nearest (inside) and furthest (outside) from the viewer, blue below red and green below blue. The double crossover molecule on the right corresponds to the two helices nearest the bracket, rotated as indicated by the coordinate system markers. Crossovers strands between helices are indicated in this view for the red helices. Those for the blue and green helices are 120° away from there and are not shown. However, they are evident in the diagram on Figure 14e.

This system was remarkably simple, but it produced crystals. We obtained scattering at the synchrotron (line X25) that indicated that we had generated both the proper symmetry, $(00l)$ was systematically absent unless $l = 3n$, and the predicted cell dimensions, $a = b = 34.5 \text{ \AA}$ observed vs. 33.75 \AA predicted and $c = 360 \text{ \AA}$ observed vs. 355 \AA predicted. Sadly, the resolution is about 15 \AA at this time, possibly because the crystals have been shocked osmotically by cryosolvents,

and possibly because the solution value of ~ 10.5 nucleotide pairs per turn is sufficiently different from the solid-state value of ~ 10.0 nucleotide pairs per turn as to cause massive disruptions of the structure over 35 nucleotides. The former was one of the problems, and we tried other conditions and equilibration protocols. The latter was also a problem and we changed the number of nucleotides in the repeat unit. We also tried more units to get closer to the 'magic' numbers that we may need to make molecular helicity commensurate with exact periodicity.

We also tested other motifs that are much more spacious than the DX, for example the DNA parallelogram. In appropriate conditions, a single crossover molecule stacks its four arms into two helical domains [CTN+88]. These are usually disposed $\sim 60^\circ$ from antiparallel [MSS99], but they can also be $\sim 70^\circ$ from parallel if appropriate analog backbones are used [SLM+00]. Although somewhat floppy as individual junctions, four of them can be combined into a useful and robust parallelogram structural building block. This concept is illustrated schematically on Figure 15a, and an AFM picture illustrating the tunable cavities produced by this motif is shown in Figure 15b. Each of the two helix pairs (red or blue) represents a system similar to the two helices of a DX motif. Consequently, it seems likely that we could build the same lattice with the parallelogram that we can build with the DX molecule. Indeed, we have done so, producing very large crystals (>1 mm in the longest direction). One difference between the DX system and the parallelogram system is that the helices in the DX are antiparallel, whereas they are parallel in the parallelogram. The lattice formed with this system was much more commodious towards potential guest molecules than the lattices discussed above.

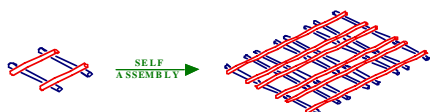


Figure 15a

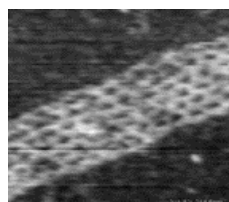


Figure 15b

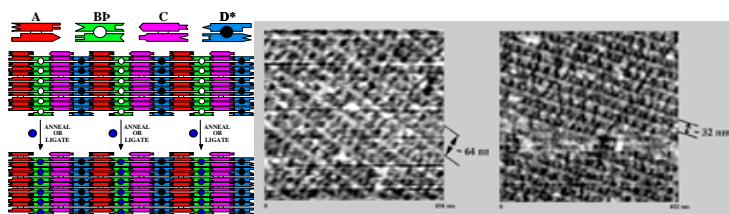


Figure 15d

Figure 15d

Figure 15e

Figure 15: The design of 3D DNA Lattices

We also designed a DX lattice based on the assumption that DNA is 10.4 fold. This led to a tetragonal arrangement in which 13 ($10.4 * 5/4$) nucleotide pairs are placed between successive units. We have preliminary crystals of this lattice as well. How can we insert hetero-molecules

into such lattices? We have demonstrated already in 2D that we can add a DNA molecule to a sticky end in a periodic system [Liu99]. This is illustrated in Figures 15c-15e.

The schematic in Figure 15c illustrates a 2D lattice in which a sticky end is represented by a white filled circle. A DNA hairpin is added by ligation or simple hydrogen bonding to produce the new pattern on the bottom left. Figure 15d is an AFM image of a lattice containing a hairpin (like D* on the left) and a hairpin (like B° on the left). The two features alternate, being separated by 32 nm, with an overall periodicity of 64 nm. The hairpin is the more intense feature. Figure 15e shows the same lattice after a hairpin has been added to the sticky end by complementarity. All rows are equivalent, with a spacing of 32 nm. Thus, it was possible to add material to a DNA lattice. We utilized this methodology to add nanocrystals from the Alivisatos laboratory to 2D lattices.

3.4.3 Experimental Demonstration of 3D DNA lattices diffracting to ~ 7.5 Å (NYU).

In the Seeman lab at NYU, the goals for the project were largely devoted to defining DNA motifs that are compatible with 3D self-assembly. We designed two different motifs that produce some extent of X-ray diffraction, a TX motif diffracting to ~ 7.5 Å and a DX motif diffracting to ~ 8 Å.

We suspected that the problems with these crystals result from DNA helicities incommensurate with the design. We screened molecules with varied helicities to solve this problem. By themselves, screening helicities did not solve the problem, although we have been able to produce a variety of non-diffracting solids.

In a new direction, we also pursued strategies to protect ourselves from tile writhe, as well as to establish more precisely the structures of the tiles. We also altered crystallization protocols to include thermal as well as precipitant crystallizations. We suspected that the lack of diffracting material from thermal protocols was due to the presence of incompletely formed molecules in solution at the time of solidification. Melting studies of both species (tiles and crystals) confirmed this hypothesis. In addition, the first cooling displayed hysteresis, but the second did not. We are using this fact to improve cooling protocols.

We explored other 3D designs, such as a triangle-like motif that spans 3-space. We obtained diffraction to ~ 11 Å, but the motif is not well-behaved. We also generated the first trigonal 2D lattice, using a new DX triangle motif and extended this motif to 3D.

3.4.4 *Securing Guests.*

The problem in 3D is more difficult for crystallographic applications. Guests can be tied down to the components of the host lattice before it is assembled. Potential blockage of the host lattice by successfully bound guests near the outside is the reason for this strategy. DNA-DNA interactions are appealing, at least in part. A guest covalently linked to a polypyrimidine tract can be readily bound by polypurine tracks on the host, creating a triple helical structure based on Hoogsteen base pairing [Hoo59]. Uniqueness and phasing can be achieved by inserting cytidines at prescribed loci along the tether. As a Cytosine, C is moved along the tethering strand in a series of molecules, it binds to the same Guanine Cytosine, GC pair with greater tautness, as illustrated in Figure 16a. This diagram shows a macromolecule tethered with three strands, but the details of only one are shown. Of course, cytidine forms triple helices only at acid pH, which is inconvenient, and may adversely affect the guest molecule. Fortunately, the cytidine analog

[YZS+01]. The bottom of Figure 16b indicates the test system where switching states alternates between a 'cis' configuration (PX) and a 'trans' configuration (JX₂). The bottom of Figure 16c shows AFM pictures of four successive states through this system.

Although this device might also power a nanomechanical motor, we see its value as a means to produce a large number of structural states. If N of these devices (distinguished by the control sequences (green and purple in Figure 16b) were to be incorporated into a lattice, we could produce 2^N distinct structural states, leading to a DNA version of nanorobotics. This capability could lead to a nanoscale assembly line for the production of molecules otherwise not attainable. We have pointed out elsewhere [See00a] that the positional synthesis advocated by many nanotechnology enthusiasts [e.g. Dre86] has only limited applications because systems eventually go to their thermodynamic minima. However, in cases where potentially reactive sites are chemically identical, but spatially different (e.g., the atoms on the walls of a carbon nanotube), positional addressability has merit.

An outline for a nanofactory device is shown in Figure 16d. This drawing depicts a 'nanofactory', in which a series of PX/JX₂ devices are shown going through three cycles of configurations. The bottom of each panel contains a set of 3 augmented device molecules mounted on a lattice. At the top of each panel are set strands, controlled electronically, by a Nanogen device (ideally), which has electrodes mounted under hybridized strands and which can propel them into solution in response to a signal. The set strands on the device are labeled P(urple)-up and G(reen)-up. In cycle 1, the three G-up set strands are still on the device, and the P-up set strands have been released into solution. Inspection of the circles in molecules 1, 2 and 3 in the bottom panel of cycle 1 shows that the purple circles are above the green circles (hence, P-up). In cycle 2, the G-up set strands for molecules 1 and 3 have been released, and the P-up set strand for molecule 2 has been released. In cycle 3, the P-up set strands for molecules 1 and 3 have been released, and the G-up set strand for molecule 2 has been, establishing the configurations shown at the bottom of the panel. Thus, the basis for a set of configurations, leading to nanorobotic manipulations has been developed.

3.5.2 Shape-Change Induced Assembly of 3D DNA Nanostructures from 2D DNA lattices (Reif).

We investigated a method for assembly of 3D DNA nanostructures from 2D DNA lattices by applying restriction enzymes to targeted sections of a regular self-assembled 2D DNA lattice [Liu99], and in the resulting fragmented DNA lattice inducing a sequence of shape changes by either the nano-mechanical transducer devices of Seeman and Yurke or environmental changes (Figure 17). There is now a vast body of practical engineering developed in the construction of MEMS devices from silicon substrates that might be brought to bear and leveraged to develop techniques for the multi-step assembly of 3D DNA nanostructures from 2D DNA lattices. These devices are first fabricated within a 2D domain via optical lithography, and then subsequent phases that exploit additional E-beam lithography, chemical release processes, and/or simple environmentally dependant shape changes (electrostatic or temperature based) to fully assemble the resulting MEMS devices in 3D. For example, a box-shaped device (with an open top) can be assembled by fabricating the bottom square and also the four sides adjoining the bottom square,

and then forcing (e.g., via environmentally dependent shape changes) the sides upward, and locking these sides together via simple mechanical latches.

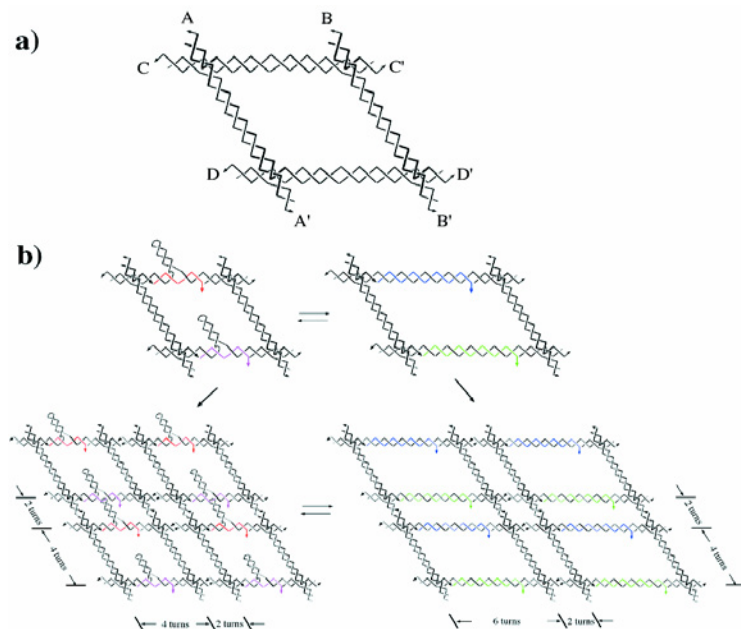


Figure 17A: Schematic drawing of the two state 2D lattices actuated by DNA nano-actuator devices

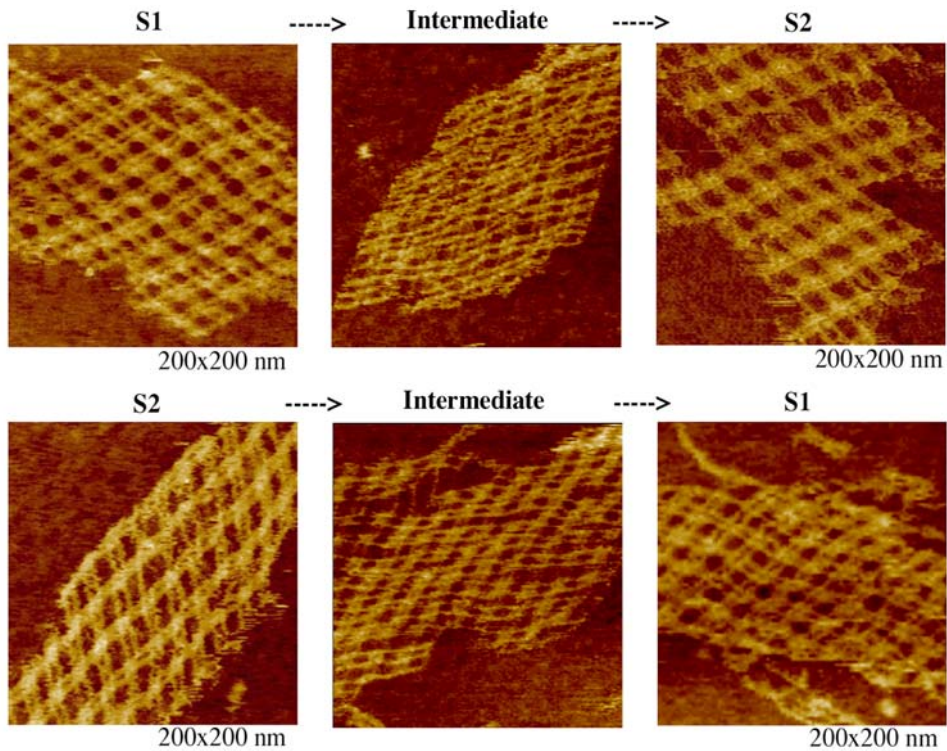


Figure 17B: AFM evidence for the two state DNA lattice actuated by DNA nano-actuator devices

We demonstrated use of shape-change to assemble complex 3D conformations of DNA lattices, from portions of 2D DNA lattices. A 3D assembly was begun with the assembly of 2D DNA lattices followed by the folding of these 2D assemblies into a 3D DNA nanostructure. In this process, we would proceed in a manner similar to the above described assembly process used in MEMS. The sequenced use of restriction enzymes (which can cut the lattices at specified locations) provides an analogue in DNA lattices to the use in MEMS of E-beam lithography and chemical release processes. Simple environmentally dependent shape changes in DNA lattices seem feasible to achieve. Moreover, the nano-mechanical transducer devices Seeman [MSS+99] first described as components of lattices (or the nano-mechanical transducer devices of Yurke [YTM+00, TYM00, SY01], known as “molecular tweezers”, based on selective use of DNA hybridization) provide for the capability of controlled flexibility in DNA lattices, where each device responds individually to a set of signals. Finally, DNA annealing and ligation can be used in place of the mechanical latches used in the MEMS field to secure the MEMS devices. Complex assemblies of 3D DNA nanostructures constructed from 2D DNA lattices may be created using a multi-step approach of this sort, where the assembly proceeds in a series of steps. We tested this approach by the assembly of 3D polygonal objects (e.g., various folded 2D lattices) from a 2D DNA lattice, by the use of restriction enzymes, and then shape changes in DNA lattices induced by nano-mechanical devices and environmental changes.

3.5.3 Nano-mechanical Walkers (Reif, Seeman)

Various molecular motors that can walk on periodic arrays were demonstrated. Mechanisms to insure that the walker stays properly oriented and moves in the correct direction along the substrate were devised (Figure 18).

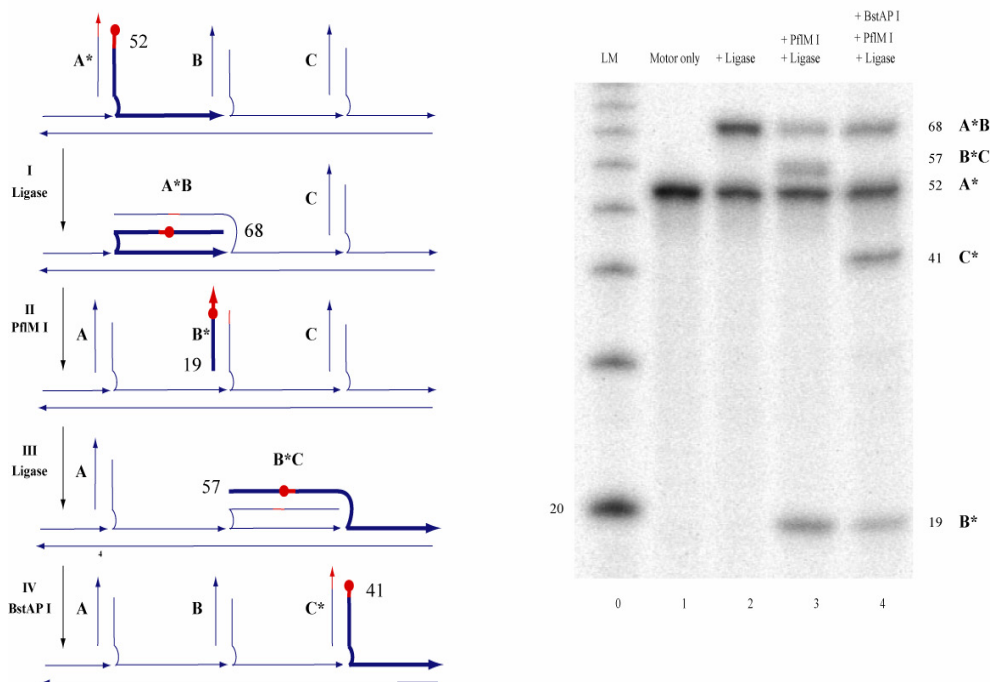


Figure 18: Autonomous Motion of the Duke DNA Walker (Duke)

The walker was based on the use of DNA enzymes. There were no external environmental changes to sequence the walker – the walker was autonomous.

A DNA Walker. (NYU)

Seeman designed and demonstrated a DNA walker using operating principles similar to those employed in the molecular tweezers [YTM00] and nanoactuators [SY01]. These used external environmental changes to sequence the walker.

3.6 Software Assisting the Design and Simulation of DNA Nanostructures (Duke & Caltech).

3.6.1 Software for Design of DNA Nanostructures and their DNA Sequences (Caltech).

As DNA computing and DNA nanotechnology become ever more sophisticated, resulting in ever more complex systems, automated design becomes essential for reliable results. Current approaches [See90] employ algorithms to minimize the potential for undesired interactions between DNA strands; however, considerable amounts of user input and geometric insight are required to produce successful designs. There are two stages to the design of DNA

nanotechnology structures: (i) going from a sketch of the desired strand topology and geometry to a choice of specific lengths of double-helical regions that minimize torsional strain on the molecules, and (ii) choosing particular oligonucleotide sequences that fold into the desired structure, and do not get stuck in alternative or intermediate folds [See85]. Nucleotide sequences for synthetic DNA structures must be carefully selected to provide both specificity and affinity for the desired interactions. As the designs increase in size and complexity, it becomes increasingly desirable to develop a systematic computational approach for DNA sequence selection – one that does not depend upon the user’s deep insight into the geometry and biochemistry of DNA, (Figure 19).

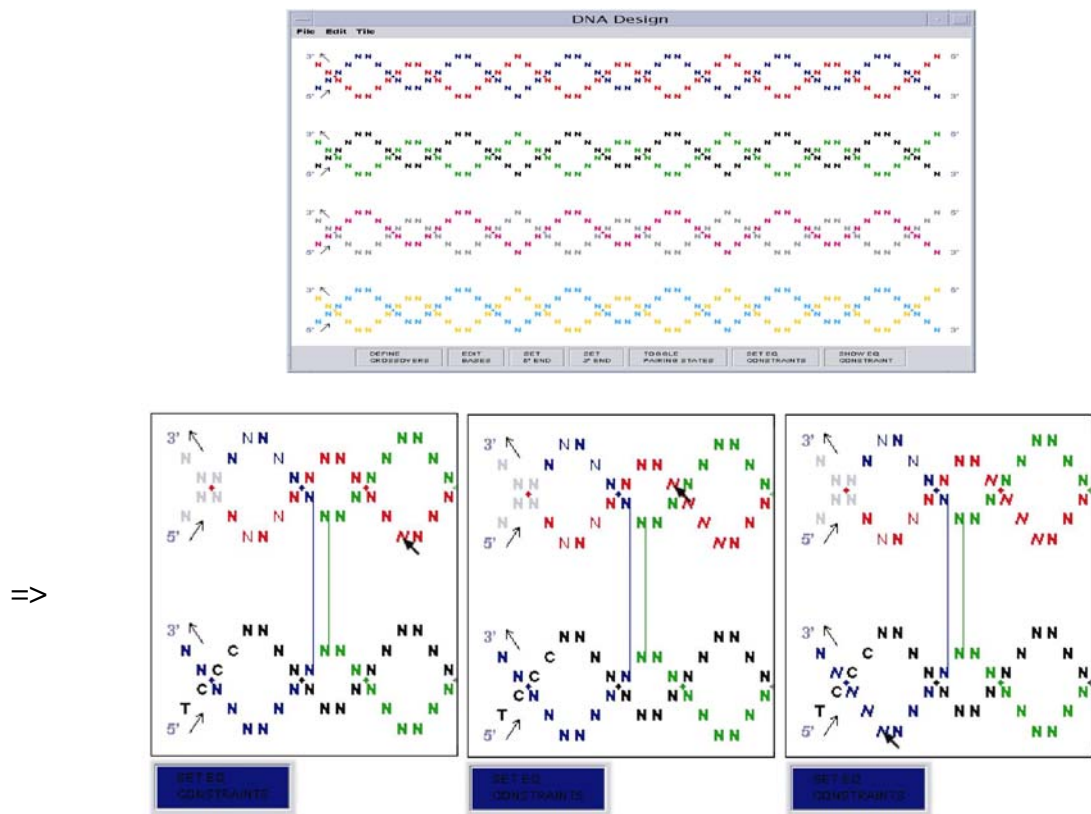


Figure 19: Software for DNA tile Design developed by Caltech & Duke University:
The user can define crossovers between helices, by clicking sequentially the two bases to be connected in 5' to 3' order. The software then designs the DNA sequences.

A popular model from the protein design community attempts to optimize the stability of a specified backbone fold by selecting the ‘rotamer’ (discrete side-chain representation) at each position that minimizes an empirical potential function over all atoms in the protein [DM97]. To minimize computational complexity, the potential function must contain at most pairwise energy terms (e.g. van der Waals interactions). Although this discrete optimization problem is NP Hard [PWprep], it is feasible to identify the global minimum energy sequence using deterministic computational methods for problems large enough to be of significant biotechnological interest

[DDHL92, DM97, PSDM00]. For DNA, the simplified alphabet and thermodynamics imply that fold specificity is the primary design consideration. A useful approach for design of DNA sequences minimizes ‘sequence symmetry’ by ensuring that ‘words’ comprised of a specified number of contiguous bases appear only once in the design [See90]. For DNA design, we found that it is conceptually possible to optimize simultaneously for affinity and specificity by defining rotamers based on DNA words, incorporating sequence symmetry minimization terms into a DNA potential function, and identifying the global minimum energy sequence using search algorithms developed for protein design. Challenges of this approach include the large number of positions of interest for DNA designs, the large number of rotamers generated by base permutations in each word, and the relative energetic similarity of the different rotamers. For the small four-letter alphabet of DNA, the dominance of Watson-Crick base pairing enables the use of thermodynamic models that are based solely on strand topology and alignment rather than three-dimensional geometry. The objective becomes the identification of a sequence that prefers the target topology over all other topologies (possibly by a specified energy gap), given a certain set of thermodynamic rules. This challenging discrete optimization problem may be approximated by various heuristic methods. We also investigated algorithms that incorporate both geometrical and thermodynamic aspects of the DNA folding and self-assembly processes.

Caltech developed a mathematical/algorithmic framework for the analysis and design of multi-stranded DNA complexes. We have also established a framework for energy calculations on multi-stranded DNA complexes, and we are initiating work coding algorithms for minimum free-energy, partition functions, and stochastic kinetic simulations. Programming of the multi-stranded algorithms is now underway (J. Shaeffer, R.M. Dirks). We have been testing our algorithms on a variety of design tasks, including tiles and nucleating structures for de-multiplexing RAM lattice and other algorithmic self-assembly systems.

Caltech performed a thorough computational evaluation of algorithms for designing nucleic acid secondary structure. Traditional approaches to rational design rely on either a positive design paradigm based on affinity optimization or a negative design paradigm based on specificity optimization. Our evaluation of several commonly used design criteria suggests that explicit implementation of both paradigms yields significantly better results than either paradigm alone. This assessment relies on a closed computational feedback loop that uses fast partition function algorithms to calculate the probability that a particular sequence adopts a target secondary structure. Probabilities close to unity indicate both high affinity and high specificity. The relative performance of design methods is robust to variations in the target structure and to perturbations in the empirical potential function parameters. Methods based on sequence symmetry minimization (N. Seeman) are widely used -- our results suggest that they could be improved by incorporating a positive design component. Optimization of equilibrium properties leads to sequences with widely differing folding times. The observed decoupling of thermodynamic and kinetic properties suggests that there are sufficient degrees of freedom in sequence space to allow the design of more complex features of the energy landscape (e.g. metastable states).

Based on prior work of Caltech, Duke continued development of software for DNA sequence design. Duke improved existing software for design of DNA nanostructures and their DNA sequences and tested that software for the design of improved triple-crossover and single-strand DNA tiles.

3.6.2 Developed the first known algorithm for computing partition functions for possibly pseudo-knotted, single-stranded DNA structures (Caltech)

This allows the calculation of the probability that a desired target structure will result for a given DNA sequence and provides an avenue for establishing both positive and negative design of DNA structures. The pseudoknot partition function algorithm has now been extended to compute the base pairing probabilities for all base pairs in the design

3.6.3 Software for Fast Simulation of DNA lattice Dynamics using Empirical Models (Duke & Caltech).

To aid the process of designing and analysis of DNA lattices, it was very useful to approximate and visualize the process of the conformational deformation of large DNA lattices at an acceptable speed. One approach to studying the dynamics of DNA lattices is by laboratory experiments via real-time AFM imaging. Due to the technical challenges involved in such experiments, we also simulated the dynamics of DNA lattices so as to predict conformations in a systematic and automatic way (to predict conformations, simulation is then performed based on this model until near equilibrium is reached). An appropriate mathematical modeling of DNA lattices was the first step to achieve the goal. Various approaches for DNA dynamics have been developed, with different degrees of accuracy and simplicity. Such studies range from the high-resolution quantum mechanics, to molecular mechanics [BR94], and finally to empirical models [Sch74, Sch80, AM84, Sch85, CL94, HGAS96] (e.g., continuous flexible rod models). Lower resolution (simpler) models generally allow larger time steps and less computation required at each step, thus faster simulation, while more complicated models potentially provide better and more accurate simulation.

Unfortunately, the software simulation of large DNA lattices containing hundreds of DNA tile nanostructures was computationally challenging using conventional molecular dynamics simulation software. We circumvented this computational difficulty by developing fast, scalable DNA molecular dynamic simulation software. The software employed an empirical model for DNA dynamic simulation at base-pair level.

Empirical Models for DNA. A discrete modeling of DNA at the base-pair (b-pair) level was first discussed by Schellman [Sch74, Sch80] to capture certain thermodynamic aspects of DNA molecules. His preliminary model treated each base-pair as a virtual bond (or a bead), and a virtual axis composed of the chain of virtual bonds was formed, so the bending energy of the chain is taken into account. The DNA b-pair chain was modeled as a continuous elastic rod with certain radius. His model was later modified and improved in many aspects [AM84, Sch85, CL94, HGAS96]. For example, to model the DNA base-pair chain as a continuous elastic rod, and to include twist explicitly or implicitly, the twisting energy for each base-pair around the axis was later also included, as were other energy terms. The underlying idea of such approaches is to describe the macroscopic shape of DNA molecules with the help of local parameters based on the base-pairs. To be more specific, dsDNA strands bend, twist, and stretch in response to base sequence and to specific interactions with the chemical environment. The idea is to extract a DNA model with local energy components, where the coefficients in the model come from empirical results of known DNA X-ray crystallographic literature.

In our simulation software, a series of refined models incorporating known empirical results on

angular and translational parameters (distortion and spring constants) between adjacent base-pair planes (namely, twist, roll, tilt, shift, slide, and rise) were applied to compute the conformation and dynamics of double helical DNA. The model was also refined to incorporate base sequence-dependent variations in these parameters. Previous models only attempted to model dsDNA, RNA, etc., rather than DNA nanostructures. One key innovation was the explicit modeling of Holliday junctions, which have not previously been modeled by these techniques, and will require special parameters as well as the modeling of their geometry. Our resulting model was less accurate than known dynamic simulation approaches at the atomic level. Nevertheless, the simulation results were reliable enough to provide important insight on the dynamics of large DNA lattices. Moreover, the performance of this simulation approach scaled linearly with the number of DNA bases of the DNA lattice, so simulations of very large DNA lattices were feasible. A software tool was developed based on this algorithm to allow the user to visualize the dynamics. Reif has already tested this approach on several dsDNA molecules, and performance is encouraging. Our software extended to single Holliday junctions, then to single DNA tiles incorporating multiple Holliday junctions, and finally to large DNA lattices containing between a few hundred and a few thousand DNA tiles.

4 CONCLUSIONS

4.1 Why the DNA Nanotechnology Approach Provided a Unique Opportunity for Technology Transfer.

DNA nanostructures present a unique opportunity for technology transfer. The DNA nanotechnology approach outlined in the 1980's to rational macromolecular crystallization [See82] and to 3D integrated nanoelectronics [RS87] remains the approach that is richest in information content. This information content translates directly into the greatest amount of control over processes, leading to self-assemblies that are controlled by design, rather than random. It is certainly possible to crystallize macromolecules by trial and error, but many studies have waited years for adequate crystals. Likewise, many stable crystals decompose when interesting ligands (inhibitors, effectors, drugs) are added to the crystals. In the system described here, the target of study was, for the first time, not be involved in lattice contacts, so that guest distortions caused by binding the ligand do not imperil the stability of the crystal. In a similar vein, nanoelectronic components can be crystallized but they cannot be organized into circuits that require nanoscale features. In summary, this was the first system that offered a rational approach to crystallization and also to complex 3D nanoelectronic assembly on the massively parallel scale typical of molecular systems. Nanorobotic components are also available from this approach, leading to previously unattainable 3D spatial control over chemical reactivity.

The funded work provided the fundamental science experiments to test out applications in crystallization, molecular electronics and robotics. Current and future research provides for expansion of DNA nanotechnology by combining with exciting recent advances in peptide, protein, RNA, and carbon nanotube engineering. These research programs enhance the feasibility and pace of technology transfer to widespread application of DNA lattices in crystallization,

molecular electronics and robotics. We directly involved various commercial start-up companies (e.g., Molecubotics Inc. and Quantum Dot Corporation) in our research.

4.2 Overview of Applications That Can be Commercialized.

The ability to assemble nanoparticles in a precise and controlled way is key to the fabrication of a variety of nanodevices. Networks of nanometer-sized metal or semiconductor islands, or quantum dots, may exhibit a variety of quantum phenomena, with applications in optical devices [BG98], nanometer-sized sensors [CR98], advanced computer architectures [Li99a], ultra dense memories [Li99b], signal classification [BW00], and quantum-information science and technology [Li93, Be00, Di00].

4.2.1 Applications of Programmable DNA nanotechnology.

DNA nanotechnology developed in this contract enables a wide range of commercial applications where precise programmable control of molecular events and structures is necessary. Initial applications are likely to be crystallization, nanoscale patterning for electronic circuits, biochips, and biosensors. Applications include new molecularly precise “smart” materials, optical materials, and molecular motors. Ultimately, nanotechnology, including the DNA nanotechnology developed here, will leave very few industries un-transformed.

4.2.2 Applications of “Molecular Building Blocks” with Tailored Functionalities.

DNA nanotechnology applications have in common that at their core, they require an extensible set of “molecular building blocks” (MBBs), based mostly on DNA tiles that may carry some additional functionalities useful in a given product, and a general assembly method, controlled by DNA hybridization, which can arrange these MBBs into a wide variety of complex structures. The results of this research effort allow routine assembly of dozens or hundreds of building blocks, which form the foundation and chassis for mounting active components. Currently, no other general purpose methods exist for achieving this goal, and so this research was a major step forward in enabling novel kinds of products.

4.2.3 Other Applications of Research.

The patterning of preformed nanotubes may be useful for fabrication of structures with novel electronic or physical properties. Possible future uses for kinetically-active protein devices (immobilized on DNA lattices) of might include molecular conveyor-belts, sensor-less sorting of nanometer scale objects, pumping surfaces for facilitated flow in MEMS, and transducers of coordinated motion in nanomachines or nanobots. We were able to organize proteins in 2D on self-assembled DNA lattices, and in the future these can be used to accomplish specific mechanical tasks. Elastic Like Proteas (ELP) Peptides can be engineered with important molecular properties such as chain length, (directly related to nanoactuator stroke), to be precisely controlled, and hence are especially attractive for application as molecular components for nanoactuators. They may be used to provide unique materials with controllable shape: e.g., repeated layers of DNA sandwiched between layers of ELP will result in a material whose overall length will alter significantly when the ELP undergoes its conformational change.

4.3 Intellectual Property.

A patent on periodic DNA lattices in 2D and 3D was submitted jointly by NYU and Caltech at the time that [WWS98] approved in March, 2001. A patent application by Seeman on the PX-JX₂ device is in the process of being prepared by patent attorneys working for NYU. Further patent protections were sought as feasible.

4.4 Commercialization and Start Ups.

This research resulted in various scientifically savvy companies poised to commercially exploit the frontier advances in DNA nanotechnology.

4.4.1 Molecubotics Inc.

Winfree has worked actively with Molecubotics Inc to commercialize some of the results of this research effort. By visits to our laboratories, Winfree has trained Molecubotics personnel about our research and technical expertise. Molecubotics were therefore an active partner in the development of application-oriented research developed here, especially the step-wise assembly techniques in which they will take the lead.

4.4.2 Quantum Dot Corporation.

The DNA/nanocrystal conjugates being developed here can be used as building blocks for complex materials and electrical and optical devices. Also, the conjugates themselves are extremely useful in a wide variety of biological labeling and quantification problems. The nanocrystals can be readily detected optically, electrically, or magnetically, and thus provide clear signatures for the presence of a particular oligonucleotide. Applications for this range from expression library screening to detection of pathogens. The technology is so important that several start ups have emerged recently to exploit the use of nanocrystals for biological detection. One of these, Quantum Dot Corporation (www.qdots.com) has spun off from the Berkeley nanocrystal program, and already employs 60 people.

4.5 Involvement of Venture Capitalists For Planning Further Commercialization and Start Ups.

Reif and Seeman have been in touch with three venture capitalists,

- David J. O'Reilly of Iconic Pharmaceuticals, Inc.
- Clay B. Thorp, of Catalysta, Inc. URL: www.catalystaventures.com and
- John Monahan monahan@vitasoft.org

to discuss the possibilities of commercialization of this technology. Mr. O'Reilly shared the view that the crystallographic applications of DNA nanotechnology are likely to be the first to be of commercial value. Both of the other venture capitalists, as well as Reif and Seeman, feel that this will be followed by applications in molecular electronics.

5 REFERENCES

- [AJP+96] Alivisatos, A. P.; Johnson, K. P.; Peng, X. G.; Wilson, T. E.; Loweth, C. J.; Bruchez, M. P.; Schultz, P. G., "Organization of Nanocrystal Molecules Using DNA," *Nature*, 382, 609-611, (1996)
- [Be00] S. Benjamin, "Schemes for parallel quantum computation without local control of qubits," *Phys. Rev. A* 61, 020301(R), (2000)
- [BG98] D. Bimberg, M. Grundman, N. N. Ledentsov, *Quantum Dot Heterostructures* (John Wiley & Sons, New York, NY, 1998).
- [BMG+98] Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P., "Semiconductor nanocrystals as fluorescent biological labels," *Science*, 281, 2013-2016, (1998)
- [BR94] D. L. Beveridge and C. Ravishanker, Molecular dynamics studies of DNA, *Curr. Opin. Struct. Biol.*, 4, 246-255, (1994)
- [BRW05] Rob D. Barish, Paul W. K. Rothmund, and Erik Winfree, Two Computational Primitives for Algorithmic Self-Assembly: Copying and Counting, , to appear 2005.
- [BW00] J. Barhen, J.C. Wells, V. Protopopescu, and Y. Braiman, "Neuromorphic Pattern Recognition Using Arrays of Quantum Dots", *Proceedings of the World Multiconference on Systemics, Cybernetics, and Informatics (SCI-2000)*, July 23-26, 2000, Orlando, Florida, Vol. XI, p.257
- [BN98] Brown, K.R. and M.J. Natan, Hydroxylamine seeding of colloidal Au Nanoparticles in solution and on surfaces. *Langmuir*, 14, 726-728, (1998)
- [CMC+04] A. Carbone, C. Mao, P. Constantinou, B. Ding, J. Kopatsch, W. B. Sherman and N.C. Seeman, 3D Fractal DNA Assembly from Coding, Geometry and Protection, *Natural Computing* 3, 235-252, (2004)
- [CS02a] A. Carbone and N.C. Seeman, Circuits and Programmable Self-Assembling DNA Structures, *Proc. Nat. Acad. Sci. (USA)* 99 12577-12582, (2002)
- [CS02b] A. Carbone and N.C. Seeman, Fractal Designs Based on DNA Parallelogram Structures, *Natural Computing* 1, 469-480, (2002)
- [CS03] Carbone and N.C. Seeman, Coding and Geometrical Shapes in Nanostructures: a Fractal DNA-Assembly, *Natural Computing* 2, 133-151, (2003)
- [CS04] A. Carbone & N.C. Seeman, Molecular Tiling and DNA Self-Assembly, *Aspects of Molecular Computing, Lecture Notes in Computer Science* 2340, Springer-Verlag, Berlin, 61-83, (2004)
- [CC79] Carter, C.W., Jr. & Carter, C.W., Protein Crystallization Using Incomplete Factorial Experiments, *J. Biol. Chem.* 254, 2219-2223, (1979)
- [CST98] A. M. Cassell, W. A. Scrivens, J. M. Tour, *Angew. Chem. Int. Ed.* 37, 1528 (1998).
- [CS91] J. Chen and N.C. Seeman, The Synthesis from DNA of a Molecule with the Connectivity of a Cube, *Nature* 350, 631-633, (1991)
- [CCC+99] Chin TM, Chung KY, Chen JJ, Lin WC, Kan LS, The synthesis of N-4-(6-aminopyridin-2-yl)-2'-deoxycytidine for recognizing the CG base pair at neutral pH by oligodeoxyribonucleotide-directed triple helix formation, *J. Chin. Chem. Soc.* 46, 751-758, (1999)

- [CL94] G. Chirico and J. Langowski, Kinetics of DNA supercoiling studied by Brownian dynamics, *Biopolymers*, 34, 415-433, (1994)
- [CTN+88] M.E.A. Churchill, T.D. Tullius, N.R. Kallenbach and N.C. Seeman, A Holliday Recombination Intermediate is Twofold Symmetric. *Proc. Nat. Acad. Sci. (USA)* 85, 4653-4656 (1988)
- [CR98] A. N. Cleland, M. L. Roukes, "A Nanometre-Scale Mechanical Electrometer" *Nature* 392, 160, (1998)
- [CBP96] J. L. Coffey, S. R. Bigham, X. Li, R. F. Pinizzotto, Y. G. Rho, R. M. Pirtle, I. L. Pirtle, *Appl. Phys. Lett.* 69, 3851, (1996)
- [CRW04] Matthew Cook, Paul W.K. Rothmund, and Erik Winfree. Self-Assembled Circuit Patterns. *DNA Computers 9*, LNCS, 2943, 91-107, (2004)
- [DM97] Dahiyat, B.I. and Mayo, S.L., *De novo* protein design: fully automated sequence selection. *Science* 278, 82-87, (1997)
- [DDHL92] Desmet, J., De Maeyer, M., Hazes, B., and Lasters, I., The dead-end elimination theorem and its use in protein side-chain positioning. *Nature*, 356, 539-542, (1992)
- [DS00] M. H. Devoret and R. J. Schoelkopf, "Amplifying quantum signals with the single-electron transistor," *Nature*, vol. 406, 1039 - 1046, (2000)
- [DSS04] A. Ding, R. Sha & N.C. Seeman, Pseudohexagonal 2D DNA Crystals from Double Crossover Cohesion, *J. Am. Chem. Soc.* 126, 10230-10231, (2004)
- [DLW+04] Robert M. Dirks, Milo Lin, Erik Winfree and Niles A. Pierce. Paradigms for Computational Nucleic Acid Design. *Nucleic Acids Research*: 32(4):1392-1403, (2004)
- [Di00] D. P. DiVincenzo, "The physical Implementation of Quantum Computation," *Fortschr. der Phys.* 48, 771, (2000)
- [DY98] Dogterom, M. and Yurke, B. Microtubule dynamics and the positioning of microtubule organizing centers, *Phys. Rev. Lett.* 81, 485-488, (1998)
- [DDE96] M. S. Dresselhaus, G. Dresselhaus, and P. C. Eklund, *Science of Fullerenes and Carbon Nanotubes*. San Diego: Academic Press, (1996)
- [Dre86] K.E. Drexler, *Engines of Creation*, Anchor, New York, (1986)
- [DG92] A. Ducruix & R. Giegé, *Crystallization of Nucleic Acids and Protein: A Practical Approach* IRL Press, Oxford, (1992)
- [ESM+97] Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A., "Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles," *Science*, 277, 1078-1081, (1997)
- [FPR+03] Liping Feng, Sung Ha Park, John H. Reif, and Hao Yan, A Two-State DNA Lattice Switched by DNA Nanoactuator, *Angewandte Chemie [International Edition]*, 42, 4342-4346, Sept. (2003)
- [GLR00] Gehani, A., T. H. LaBean, and J.H. Reif, DNA-based Cryptography, 5th DIMACS Workshop on DNA Based Computers, MIT, June, 1999. *DNA Based Computers*, V, DIMACS Series in Discrete Mathematics and Theoretical Computer Science, (ed. E. Winfree), American Mathematical Society, (2000)
- [GLR04] A. Gehani, T. H. LaBean, and J.H. Reif, DNA-based Cryptography, *Proc. DNA Based Computers V*: Cambridge, MA, June 14-16, 1999. Published in DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Volume 54, edited by E. Winfree and D.K. Gifford, American Mathematical Society, Providence, RI, 233-249, (2000)

- [GR99] A. Gehani and J.H. Reif, Microflow Bio-Molecular Computation, 4th DIMACS Workshop on DNA Based Computers, University of Pennsylvania, June, 1998. DNA Based Computers, IV, DIMACS Series in Discrete Mathematics and Theoretical Computer Science, (ed. H. Rubin), American Mathematical Society, 1999. Also appeared in a special issue of Biosystems, Journal of Biological and Informational Processing Sciences, Vol. 52, Nos. 1-3, (ed. By L. Kari, H. Rubin, and D. H. Wood), 197-216, (1999)
- [HGAS96] P. J. Heath, J. A. Gebe, S. A. Allison and J. M. Schurr, Comparison of analytical theory with Brownian dynamics simulations for small linear and circular DNAs, *Macromolecules*, 29, 3583--3596, (1996)
- [Hoo59] Hoogsteen, K., The Structure of Crystals Containing a Hydrogen Bonded Complex of 1-Methylthymine and 9-Methyladenine, *Acta Cryst.* 12, 822-832, (1959)
- [JLS04] N. Jonoska, S. Liao, & N.C. Seeman, Transducers with programmable input by DNA Self-assembly, Aspects of Molecular Computing, Lecture Notes in Computer Science 2340, Springer-Verlag, Berlin, 219-240, (2004)
- [JSS03] N. Jonoska, P. Sa-Ardyen and N.C. Seeman, Computation by Self-Assembly of DNA Graphs, *Journal of Genetic Programming and Evolvable Machines* 4, 123-137, (2003)
- [LWR99] T. H. LaBean, E. Winfree, and J.H. Reif, Experimental Progress in Computation by Self-Assembly of DNA Tilings, 5th DIMACS Workshop on DNA Based Computers, MIT, June, 1999. DNA Based Computers, V, DIMACS Series in Discrete Mathematics and Theoretical Computer Science, (ed. E. Winfree), American Mathematical Society, (2000)
- [LWR00] T. H. LaBean, E. Winfree, and J.H. Reif, Experimental Progress in Computation by Self-Assembly of DNA Tilings, Published in DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Volume 54, edited by E. Winfree and D.K. Gifford, American Mathematical Society, Providence, RI, pp. 123-140, (2000)
- [LYK+00] T. LaBean, H. Yan, J. Kopatsch, F. Liu, E. Winfree, J.H. Reif and N.C. Seeman, The Construction of DNA Triple Crossover Molecules, *Journal of the American Chemical Society* 122, 1848-1860, (2000)
- [LYP+05] Thomas H. LaBean, Hao Yan, Sung Ha Park, Liping Feng, Peng Yin, Hanying Li, Sang Jung Ahn, Dage Liu, Xiaojun Guan, and John H. Reif, Overview of New Structures for DNA-Based Nanofabrication and Computation, Invited paper to appear in *Information Sciences*, (2005)
- [LPS+04] J. D. Le, Y. Pinto, N.C. Seeman, K. Musier-Forsyth, T. A. Taton and R.A. Kiehl, Self-Assembly of Nanoelectronic Component Arrays by In Situ Hybridization to 2D DNA Scaffolding, *NanoLetters* 4, 2343-2347, (2004)
- [LAD01] B Li, D.O.V. Alonso, V Daggett. *J Mol. Biol.* 305, 581-592, 2001.
- [LPR+04] Li, H.; Park, S. H.; Reif, John H.; LaBean, T. H.; Yan, H., DNA-Templated Self-Assembly of Protein and Nanoparticle Linear Arrays, *Journal of American Chemistry Society (JACS)*, Vol. 126, No. 2, pp. 418-419, (2004)
- [LLWW01] Y. Li, J. Liu, Y. Q. Wang, and Z. L. Wang, "Preparation of monodispersed Fe-Mo nanoparticles as the catalyst for CVD synthesis of carbon nanotubes," *Chemistry of Materials*, 13, 1008-1014, (2001)
- [LS04] S. Liao and N.C. Seeman, Translation of DNA Signals into Polymer Assembly Instructions, *Science* 306, 2072-2074, (2004)

- [Li99a] K. K. Likharev, "Single-electron devices and their applications," *Proc. IEEE* 87, 606 (1999)
- [Li99b] K. K. Likharev, "New Prospects for Silicon-Based Terabit Memories and Data Storage Systems", *Nanotechnology* 10, 159, (1999).
- [Liu99] Liu, Furong, et al., "Modifying the surface features of two-dimensional DNA crystals." *Journal of the American Chemical Society* 121: 917-922, (1999).
- [LRL04] Dage Liu, John H. Reif, Thomas H. LaBean, DNA Nanotubes, Construction and Characterization of Filaments Composed of TX-tile Lattice. DNA Based Computers (DNA8), Sapporo, Japan, June 10-13, 2002, (Edited by Masami Hagiya and Azuma Ohuchi), Lecture Notes in Computer Science, No. 2568, Springer-Verlag, New York, (2003), pages 10-21. Published as DNA nanotubes self-assembled from triple-crossover tiles as templates for conductive nanowires, Proceedings of the National Academy of Science(PNAS), Vol. 101, No. 3, pp 717-722, (2004)
- [LC99] J. Liu, M. J. Casavant, M. Cox, D. A. Walters, P. Boul, W. Lu, A. J. Rimerberg, K. A. Smith, D. T. Colbert, and R. E. Smalley, "Controlled deposition of individual single-walled carbon nanotubes on chemically functionalized templates," *Chemical Physics Letters*, 303, 125-129, (1999)
- [LR98] J. Liu, A. G. Rinzier, H. J. Dai, J. H. Hafner, R. K. Bradley, P. J. Boul, A. Lu, T. Iverson, K. Shelimov, C. B. Huffman, F. Rodriguezmacias, Y. S. Shon, T. R. Lee, D. T. Colbert, and R. E. Smalley, "Fullerene Pipes," *Science*, 280, 1253-1256, (1999 [LI93] S. Lloyd, "A Potentially Realizable Quantum Computer," *Science* 261, 1569, (1993)
- [LCP+99] Loweth, C. J.; Caldwell, W. B.; Peng, X. G.; Alivisatos, A. P.; Schultz, P. G., "DNA-based assembly of gold nanocrystals," *Angew. Chem.-Int. Edit.*, 38, 1808-1812, (1999)
- [LMS04] P.S. Lukeman, A. Mittal, & N.C. Seeman, Two Dimensional PNA/DNA Arrays: Estimating the Helicity of Unusual Nucleic Acid Polymers, Chemical Communications 2004, 1694-1695, (2004).
- [MLR+00] Mao, C., T.H. LaBean, J. H. Reif, and N.C. Seeman, Logical Computation using algorithmic self-assembly of DNA triple-crossover molecules, *Nature*, Sept 28, 493-495, (2000)
- [MLM+05] F. Mathieu, S. Liao, C. Mao, J. Kopatsch, T. Wang, and N.C. Seeman, Six-Helix Bundles Designed from DNA, *NanoLetters* 5, 661-665, (2005)
- [MMS01] Mathieu, F., Mao, C. & Seeman, N.C., Six-Helix Bundle DNA Nanotubes (in preparation).
- [MLR+00a] C. Mao, T. LaBean, J.H. Reif and N.C. Seeman, Logical Computation Using Algorithmic Self-Assembly of DNA Triple Crossover Molecules, *Nature* 407, 493-496 (2000).
- [MLR+00b] C. Mao, LaBean, T.H. Reif, J.H., Seeman, Logical Computation Using Algorithmic Self-Assembly of DNA Triple-Crossover Molecules, *Nature*, vol. 407, Sept. 28 2000, pp. 493-495; C. Erratum: *Nature* 408, 750-750, (2000)
- [MSS+99] C. Mao, W. Sun, Z. Shen & N.C. Seeman, A DNA Nanomechanical Device Based on the B-Z Transition, *Nature* 397, 144-146, (1999)
- [MSS99] C. Mao, W. Sun and N.C. Seeman, Designed Two-Dimensional DNA Holliday Junction Lattices Visualized by Atomic Force Microscopy, *J. Am. Chem. Soc.* 121, 5437-5443, (1999)

- [MSS97] C. Mao, W. Sun & N.C. Seeman, Assembly of Borromean Rings from DNA, *Nature* 386, 137-138, (1997)
- [MSA+00] Manna, L.; Scher, E. C.; Alivisatos, A. P., "Synthesis of soluble and processable rod-, arrow-, teardrop-, and tetrapod-shaped CdSe nanocrystals," *J. Am. Chem. Soc.*, 122, 12700-12706, (2000)
- [MRM+01] Mbindyo, J.K.N., Reiss, B.D., Martin, B.R., Keating, C.D., Natan, M.J., and Mallouk, T.E., *Advanced Materials*, 13, 249-254, (2001)
- [McP82] A. McPherson, Jr., *Preparation and Analysis of Protein Crystals* Wiley, New York (1982)
- [McP00] McPherson, A. et al. Atomic Force Microscopy in the Study of Macromolecular Crystal Growth. *Annual Review of Biophysics and Biomolecular Structure* 29:361-410 (2000).
- [MDZC01] DE Meyer, GH Kong, MW Dewhirst, M. Zalutsky, and A Chilkoti. *Cancer Research*, 61, 1548-1554, (2001)
- [MC99] DE Meyer and A Chilkoti. *Nature Biotechnology* 17, 1112-1115, (1999)
- [MLM+96] C.A. Mirkin, R.L. Letsinger, R.C. Mucic and J.J. Storhoff, "A DNA-Based Method For Rationally Assembling Nanoparticles Into Macroscopic Materials," *Nature* 382, 607-609, (1996)
- [M00] C. A. Mirkin, *Inorg. Chem.* 39, 2258, (2000)
- [MML99] G. P. Mitchell, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* 121, 8122, (1999)
- [MSM+98] Mucic, R. C.; Storhoff, J. J.; Mirkin, C. A.; Letsinger, R. L., "DNA-directed synthesis of binary nanoparticle network materials," *J. Am. Chem. Soc.*, 120, 12674-12675, (1998)
- [MDS91] J.E. Mueller, S. M. Du & N.C. Seeman, The Design and Synthesis of a Knot from Single-Stranded DNA, *J. Am. Chem. Soc.* 113, 6306-6308, (1991)
- [MFS+99] Mueller, D. J., Fotiadis, D., Scheuring, S., Mueller, S. A. & Engel, A., Electrostatically Balanced Subnanometer Imaging of Biological Specimens by Atomic Force Microscope, *Biophys. J.* 76, 1101-1111, (1999)
- [PBR+05] Sung Ha Park, Robert Barish, John Reif, Gleb Finkelstein, Hao Yan and Thomas LaBean, Three-Helix Bundle DNA Tiles Self-Assemble into 2D Lattice or 1D Templates for Silver Nanowires, *Nano Letters (Communication)*, Volume 5, Number 4, pp. 693-696, (2005)
- [PLB+05] Sung Ha Park, Hanying Li, Robert Barish, John Reif, Gleb Finkelstein, Hao Yan and Thomas LaBean, Self-assembled DNA nanobundles as Templated for Silver Nanowires, Submitted for publication, (2005)
- [PPA+05] Sung Ha Park, Constantin Pistol, Sang Jung Ahn, John H. Reif, Alvin R. Lebeck, Chris Dwyer, and Thomas H. LaBean, Finite-Size, Fully Addressable DNA Tile Lattices Formed by Hierarchical Assembly Procedures, accepted to appear in *Angewandte Chemie [International Edition]*, (2005)
- [PRL+05] Sung Ha Park, John Reif, Thomas LaBean and Gleb Finkelstein, Metallic Nanowires Templated on Native and Synthetic double-stranded DNA Scaffolds, Submitted for publication, (2005)
- [PYR+04] Sung Ha Park, Hao Yan, John H Reif, Thomas H LaBean, and Gleb Finkelstein, Electronic nanostructures templated on self-assembled DNA scaffolds, *Nanotechnology* 15 p. S525-S527, (2004)

- [PYR+05] Sung Ha Park, Peng Yin, John Reif, Thomas LaBean, and Hao Yan, Programmable DNA Self-assemblies for Nanoscale Organization of Ligands and Proteins, *Nano Letters* (Communication), Volume 5, Number 4, pp 729-733, (2005)
- [PWA+97] Peng, X. G.; Wilson, T. E.; Alivisatos, A. P.; Schultz, P. G., "Synthesis and isolation of a homodimer of cadmium selenide nanocrystals," *Angew. Chem.-Int. Edit. Engl.*, 36, 145-147, (1997)
- [PMY+00] Peng, X. G.; Manna, L.; Yang, W. D.; Wickham, J.; Scher, E.; Kadavanich, A.; Alivisatos, A. P., "Shape control of CdSe nanocrystals," *Nature*, 404, 59-61, (2000)
- [PN+00] Pierce, Niles, et al., "Conformational splitting: a more powerful criterion for dead-end elimination." *Journal of Computational Chemistry* 21, 999-1009, (2000)
- [PW02] Niles Pierce and Erik Winfree. Protein Design is NP-Hard. *Protein Engineering*, v15, 779-782, (2002)
- [PWprep] Pierce, N.A. and E. Winfree, Protein Design is NP Hard, in preparation.
- [PKA01] Punties, V. F.; Krishnan, K.; Alivisatos, A. P., Control of colloidal nanocrystal shape and size: the case of Cobalt, *Science*, 291, 2115-2117, (2001)
- [QDS97] H. Qiu, J.C. Dewan and N.C. Seeman, A DNA Decamer with a Sticky End: The Crystal Structure of d-CGACGATCGT. *J. Mol. Biol.* 267, 881-898, (1997)
- [Re98] J.H. Reif, Paradigms for Biomolecular Computation, First International Conference on Unconventional Models of Computation, Auckland, New Zealand, January 1998. Published in *Unconventional Models of Computation*, edited by C.S. Calude, J. Casti, and M.J. Dinneen, Springer Publishers, pp. 72-93, (1998)
- [Re99] J.H. Reif, Local Parallel Biomolecular Computation, Third Annual DIMACS Workshop on DNA Based Computers, University of Pennsylvania, June 23-26, 1997. Published in *DNA Based Computers, III, DIMACS Series in Discrete Mathematics and Theoretical Computer Science*, Vol 48 (ed. H. Rubin), American Mathematical Society, p 217-254, (1999)
- [Re99a] J.H. Reif, Parallel Molecular Computation: Models and Simulations. Proceedings: 7th Annual ACM Symposium on Parallel Algorithms and Architectures (SPAA'95) Santa Barbara, CA, July 1995, pp. 213-223. Published in *Algorithmica*, special issue on Computational Biology, Vol. 25, No. 2, 142-176, (1999a)
- [Re02a] J. H. Reif, Molecular Assembly and Computation: From Theory to Experimental Demonstrations, plenary paper, Twenty Ninth International Colloquium on Automata, Languages, and Programming (ICALP), Málaga, Spain (July 8, 2002). *Lecture Notes in Computer Science*, New York, Volume 2380, pages 1-21, (2002)
- [Re02b] J. H. Reif, DNA Lattices: A Method for Molecular Scale Patterning and Computation, special issue on Bio-Computation, *Computer and Scientific Engineering Magazine*, IEEE Computer Society, Vol. 4, No. 1, pp 32-41, (2002)
- [Re02c] J. H. Reif, The Emerging Discipline of Biomolecular Computation in the US, invited paper to the special issue on Biomolecular Computing, *New Generation Computing*, edited by Masami Hagiya, Masayuki Yamamura, and Tom Head, Vol. 20, No. 3, 217-236, (2002)
- [Re02d] John H. Reif, DNA Computation - Perspectives: Successes and Challenges, *Science*, 296: 478-479, (2002)
- [Re03] J. H. Reif, The Design of Autonomous DNA Nanomechanical Devices: Walking and Rolling DNA. *DNA Based Computers (DNA8)*, Sapporo, Japan, June 10-13, 2002, (Edited by Masami Hagiya and Azuma Ohuchi), *Lecture Notes in Computer Science*, No. 2568, Springer-

Verlag, New York, (2003), pages 22-37. Published in *Natural Computing*, DNA8 special issue, Vol. 2, 439-461, (2003)

[Re05] John H. Reif, DNA-Based Nano-Engineering: DNA and its Enzymes as the Engines of Creation at the Molecular Scale, Invited Talk, Tenth International Meeting on DNA Based Computers (DNA10), Milano, Italy, June 7-10, 2004. *Lecture Notes in Computer Science* (Edited by C Ferretti, G. Mauri and C. Zandron), Vol. 3384, Springer-Verlag, New York, (2005)

[REP+04] Paul W.K. Rothmund, Axel Ekani-Nkodo, Nick Papadakis, Ashish Kumar, Deborah Kuchnir Fygenson, Erik Winfree. Design and Characterization of Programmable DNA Nanotubes, *JACS* 126(50):16344-16353, (2004)

[RL00] J.H. Reif and T. H. LaBean, Computationally Inspired Biotechnologies: Improved DNA Synthesis and Associative Search Using Error-Correcting Codes and Vector-Quantization, Sixth International Meeting on DNA Based Computers (DNA6), DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Leiden, The Netherlands, (June, 2000) ed. A. Condon. Springer-Verlag as a volume in *Lecture Notes in Computer Science*, (2000)

[RL01] J.H. Reif and T. H. LaBean, Computationally Inspired Biotechnologies: Improved DNA Synthesis and Associative Search Using Error-Correcting Codes and Vector-Quantization, Sixth International Meeting on DNA Based Computers (DNA6), DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Edited by A. Condon and G. Rozenberg. *Lecture Notes in Computer Science*, Springer-Verlag, Berlin Heidelberg, vol. 2054, pp. 145-172 (2001)

[RLP+01] J. H. Reif, T. H. LaBean, M. Pirrung, V. Rana, B. Guo, K. Kingsford, and G. Wickham, Experimental Construction of Very Large Scale DNA Databases with Associative Search Capability, Seventh International Meeting on DNA Based Computers (DNA7), *DIMACS Series in Discrete Mathematics and Theoretical Computer Science*, Tampa, FL, June 11-13, (2001)

[RLS00] J.H. Reif, T. H. LaBean, and N.C. Seeman. "Challenges and Applications for Self-Assembled DNA Nanostructures", Invited paper, Sixth International Meeting on DNA Based Computers (DNA6), DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Leiden, The Netherlands, (June, 2000) ed. A. Condon. Springer-Verlag as a volume in *Lecture Notes in Computer Science*, (2000)

[RLP+05] J. H. Reif, T. H. LaBean, M. Pirrung, V. Rana, B. Guo, K. Kingsford, and G. Wickham, Experimental Construction of Very Large Scale DNA Databases with Associative Search Capability, Seventh International Meeting on DNA Based Computers (DNA7), Tampa, FL, June 11-13, 2001. *Lecture Notes in Computer Science*, Springer-Verlag, New York, Volume 2340, pages 231-247, (2002). Also published as John H. Reif, Michael Hauser, Michael Pirrung, and Thomas LaBean, Application of Biomolecular Computing to Medical Science: A Biomedical Database System for Storage and Retrieval of Genetic Information and Material, Chapter 3 of *Complex Systems Science in Biomedicine* (Edited by Tom Deisboeck), Kluwer-Springer Publishers, Chapter 3, pages 701-735, (2005)

[RLS01a] J.H. Reif and T. H. LaBean, and N.C. Seeman. Programmable Assembly at the Molecular Scale: Self-Assembly of DNA Lattices, Invited paper, 2001 IEEE International Conference on Robotics and Automation (ICRA2001), Seoul, Korea, ed. Lee Beom (May, 2001)

[RLS01b] J.H. Reif, T.H. LaBean & N.C. Seeman, Challenges and Applications for Self-

Assembled DNA Nanostructures, Sixth International Workshop on DNA-Based Computers, DNA 2000, Leiden, The Netherlands, (June, 2000) ed. A. Condon, G. Rozenberg. Springer-Verlag, Berlin Heidelberg, Lecture Notes in Computer Science 2054, 173-198, (2001)

[RLS01c] J.H. Reif, T.H. LaBean, and N.C. Seeman, Challenges and Applications for Self-Assembled DNA Nanostructures, Proc. Sixth International Workshop on DNA-Based Computers, Leiden, The Netherlands, June, 2000. DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Edited by A. Condon and G. Rozenberg. Lecture Notes in Computer Science, Springer-Verlag, Berlin Heidelberg, vol. 2054, pp. 173-198, (2001)

[RLS+05] John H. Reif, Thomas LaBean, Sudheer Sahu, Hao Yan, Peng Yin, Design, Simulation, and Experimental Demonstration of Self-Assembled DNA Nanostructures and DNA Motors, Unconventional Programming Paradigms: International Workshop UPP 2004, Le Mont Saint Michel, France, September 15-17, 2004. Published by Springer-Verlag Berlin Heidelberg, edited by Jean-Pierre Banâtre, Pascal Fradet, Jean-Louis Giavitto, Olivier Michel, Lecture Notes for Computer Science (LNCS) 3566, pp 166-180, (2005)

[RPW04] Paul W.K. Rothemund, Nick Papadakis, Erik Winfree. Algorithmic Self-Assembly of DNA Sierpinski Triangles. PLoS Biology 2 (12) p424, (2004)

[RS87] B.H. Robinson and N.C. Seeman, The Design of a Biochip: A Self-Assembling Molecular-Scale Memory Device. *Prot. Eng.* 1, 295-300, (1987)

[RSY06] John H. Reif, Sudheer Sahu, and Peng Yin, Complexity of Graph Self-Assembly in Accretive Systems and Self-Destructible Systems, Eleventh International Meeting on DNA Based Computers (DNA11), London, Ontario (June, 2005), Springer-Verlag, New York, NY, edited by Alessandra Carbone and Niles Pierce, Lecture Notes for Computer Science (LNCS), (2006) [[PDF](#)]. Submitted for publication, (2005).

[RSY06] John H. Reif, Sudheer Sahu, Peng Yin, Compact Error-Resilient Computational DNA Tiling Assemblies, Tenth International Meeting on DNA Based Computers (DNA10), Milano, Italy, June 7-10, 2004. Lecture Notes in Computer Science (Edited by C Ferretti, G. Mauri and C. Zandron), Vol. 3384, Springer-Verlag, New York, (2005), pp 293-307. Published as an invited chapter in "Aspects of Molecular Computing - Essays dedicated to Nadrian Seeman on the occasion of his 60th Birthday", Springer Verlag series in Natural Computing (edited by Natasha Jonoska) Lecture Notes in Computer Science(LNCS) Festschrift, (2006)

[RW00] Rothemund, Paul W. K. and Winfree, Erik. "The program-size complexity of self-assembled squares." Symposium on Theory of Computing (STOC), May 21-23, 2000. Association for Computing Machinery.

[RWB+98] Roweis, S., Winfree, E., Burgoyne, R., Chelyapov, N.V., Goodman, M.F., Rothemund, P.W.K., Adleman, L.M., A Sticker-Based Model for DNA Computation, *Journal of Computational Biology* 5, 615-629, (1998)

[SJS+03] P. Sa-Ardyen, N. Jonoska and N.C. Seeman, Self-Assembling DNA Graphs, DNA-Based Computers VIII, LNCS 2568, Springer-Verlag, Berlin, 1-9, (2003).

[SJS03] P. Sa-Ardyen, N. Jonoska and N.C. Seeman, Self-Assembling DNA Graphs, *Natural Computing*, 2, 427-438, (2003)

[SJS04] P. Sa-Ardyen, N. Jonoska and N.C. Seeman, The Construction of Graphs Whose Edges are DNA Helix Axes, *J. Am. Chem. Soc.*, 126, 6648-6657, (2004)

[SVS03] *P. Sa-Ardyen, A.V. Vologodskii and N.C. Seeman, The Flexibility of DNA Double Crossover Molecules. *Biophysical Journal* 84, 3829-3837, (2003)

- [Sch74] J. A. Schellman, Flexibility of DNA, *Biopolymers*, 13, pp 217-226, (1974)
- [Sch80] J. A. Schellman, The flexibility of DNA. I. Thermal fluctuations, *Biophys. Chem.*, 11, pp 321-328, (1980)
- [Sch67] Schrödinger, E. *What is Life* Cambridge University Press, Cambridge, 2nd Ed., (1967)
- [SLP+04] Rebecca Schulman, Shaun Lee, Nick Papadakis, and Erik Winfree. One Dimensional Boundaries for DNA Tile Self-Assembly. *DNA Computers* 9, LNCS volume 2943:108-125, (2004)
- [SW05a] Rebecca Schulman and Erik Winfree. Programmable Control of Nucleation for Algorithmic Self-Assembly. *DNA Computers* 10, LNCS volume 3384:319-328, 2005.
- [SW05b] Rebecca Schulman and Erik Winfree. Self-Replication and Evolution of DNA Crystals, *ECAL 2005*, in LNCS 3630:734-743, (2005)
- [Sch85] J. M. Schurr, Effect of anisotropic bending rigidity and finite twisting rigidity on statistical properties of DNA model filaments, *Biopolymers*, 24, pp 1233-1246}, (1985)
- [SYW05] Georg Seelig and Bernard Yurke and Erik Winfree. DNA Hybridization Catalysts and Catalyst Circuits. *DNA Computers* 10, LNCS volume 3384:329-343, (2005)
- [SZL+05] Sha, R., Zhang, X., Liao, S., Constantinou, P.E., Ding, B., Wang, T., Garibotti, A.V., Zhong, H., Israel, L.B., Wang, X., Wu, G., Chakraborty, B., Chen, J., Zhang, Y., Mao, C., Yan, H., Kopatsch, J., Zheng, J., Lukeman, P.S., Sherman, W.B. and Seeman, N.C., Motifs and Methods in Structural DNA Nanotechnology, *Proc. Intl. Conf. Nanomaterials, NANO 2005*, July 13-15, 2005, Mepco Schlenk Engineering College, Srivakasi, India, V. Rajendran, ed., pp. 3-10 (2005)
- [SB02] N.C. Seeman & A.M. Belcher, Emulating Biology: Nanotechnology from the Bottom Up, *Proceedings of the National Academy of Sciences (USA)* 99 (supp. 2), 6451-6455, (2002)
- [SDL05] N.C. Seeman, B. Ding, S. Liao, T. Wang, W.B. Sherman, P.E. Constantinou, J. Kopatsch, C. Mao, R. Sha, F. Liu, H. Yan & P.S. Lukeman, Experiments in Structural DNA Nanotechnology: Arrays and Devices, *Proc. SPIE; Nanofabrication: Technologies, Devices and Applications* 5592, 71-81, (2005)
- [See82] N.C. Seeman, Nucleic Acid Junctions and Lattices. *J. Theor. Biol.* 99, 237-247 (1982).
- [See85] N.C. Seeman, Macromolecular Design, Nucleic Acid Junctions and Crystal Formation. *J. Biomol. Struct. & Dyns.* 3, 11-34, (1985)
- [See90] Seeman, Nadrian C., "De novo design of sequences for nucleic acid structural engineering." *Journal of Biomolecular Structure and Dynamics* 8: 573-581, (1990)
- [See91] N.C. Seeman, The Construction of 3-D Stick Figures from Branched DNA, *DNA & Cell Biol.* 10, 475-486, (1991)
- [See00a] N.C. Seeman, DNA Nanotechnology: From Topological Control to Structural Control, in *Pattern Formation in Biology, Vision and Dynamics*, ed. by A.Carbonate, M.Gromov, P.Pruzinkiewicz, World Scientific Publishing Company, Singapore, 271-309. (2000)
- [See00b] N.C. Seeman, In the Nick of Space: Generalized Nucleic Acid Complementarity and the Development of DNA Nanotechnology, *Synlett* 2000, 1536-1548, (2000)
- [See00c] N.C. Seeman, Self-Assembly and Positional Control, *The Sciences* 40(6), 3-3 (2000).
- [See01] N.C. Seeman, DNA Nicks and Nodes and Nanotechnology, *NanoLetters* 1, 22-26, (2001)
- [See02a] N.C. Seeman, DNA Nanotechnology: Life's Central Performer in a New Role, *Biological Physics Newsletter* 2 (1) 2-6, (2002)

- [See02b] N.C. Seeman, It Started with Watson and Crick, But it Sure Didn't End There: Pitfalls and Possibilities beyond the Classic Double Helix, *Natural Computing* 1, 53-84, (2002)
- [See02c] N.C. Seeman, Key Experimental Approaches in DNA Nanotechnology, *Current Protocols in Nucleic Acid Chemistry*, Unit 12.1, John Wiley & Sons, New York, (2002)
- [See03a] N.C. Seeman, At the Crossroads of Chemistry, Biology and Materials: Structural DNA Nanotechnology, *Chemistry & Biology* 10, 1151-1159, (2003)
- [See03b] N.C. Seeman, Biochemistry and Structural DNA Nanotechnology: An Evolving Symbiotic Relationship, *Biochemistry* 42, 7259-7269, (2003)
- [See03c] N.C. Seeman, DNA in a Material World, *Nature* 421, 33-37, (2003)
- [See03d] N.C. Seeman, DNA: Beyond the Double Helix, *Macromolecular Symposia* 201, 237-244, (2003)
- [See03e] N.C. Seeman, Structural DNA Nanotechnology: A New Organizing Principle for Advanced Nanomaterials, *Materials Today* 6 (7), 24-29, (2003)
- [See04a] N.C. Seeman, DNA Nanostructures for Mechanics and Computing: Nonlinear Thinking With Life's Central Molecule. *NanoBiotechnology*. Editors, Chad Mirkin and Christof Niemeyer, Wiley-VCH Verlag GmbH & Co., KgaA, Weinheim, Chapter 20, pp 308-318, (2004)
- [See04b] N.C. Seeman, DNA Nanostructures for Mechanics and Computing: Nonlinear Thinking With Life's Central Molecule. *NanoBiotechnology*. Editors, Chad Mirkin and Christof Niemeyer, Wiley-VCH Verlag GmbH & Co., Weinheim, Chapter 20, pp 308-318, (2004)
- [See04c] N.C. Seeman, DNA Nanotechnology, *Encyclopedia of Supramolecular Chemistry*, Marcel Dekker, New York, 475-483, (2004)
- [See04d] N.C. Seeman. Nanotechnology and the Double Helix, *Scientific American.*, 290, (6) 64-75, (2004)
- [See05a] N.C. Seeman, From Genes to Machines: DNA Nanomechanical Devices, *Trends in Biochemical Sciences* 30, 119-235, (2005)
- [See05b] N.C. Seeman. Structural DNA Nanotechnology: An Overview. *Methods in Molecular Biology* 303: Bionanotechnology Protocols, Editors, Sandra J. Rosenthal and David W. Wright, Humana Press, Totowa, NJ, pp. 143-166, (2005)
- [SL05] N.C. Seeman and P.S. Lukeman, Nucleic Acid Nanostructures, *Reports on Progress in Physics* 68, 237-270, (2005)
- [SLM+00] Nadrian C. Seeman, Furong Liu, Chengde Mao, Xiaoping Yang, Lisa A. Wenzler, Ruojie Sha, Weiqiong Sun, Zhiyong Shen, Xiaojun Li, Jing Qi, Yuwen Zhang, Tsu-Ju Fu, Junghuei Chen and Erik Winfree. Two Dimensional and Two States in DNA Nanotechnology. *Journal of Biomolecular Structure and Dynamics*, 11 (S2): 253-262, (2000)
- [SLM+00] N.C. Seeman, F. Liu, C. Mao, X. Yang, L. A. Wenzler, R. Sha, W. Sun, Z. Shen, X. Li, J. Qi, Y. Zhang, T.-J. Fu, J. Chen and E. Winfree, Two Dimensions and Two States in DNA Nanotechnology, *Proceedings of the 11th Conversation in Biomolecular Stereodynamics*, ed. by R.H. Sarma and M.H. Sarma, Adenine Press, New York, 253-262, (2000)
- [SML+00] N.C. Seeman, C. Mao, F. Liu, R. Sha, X. Yang, L. Wenzler, X. Li, Z. Shen, H. Yan, P. Sa-Ardyen, X. Zhang, W. Shen, J. Birac, P. Lukeman, Y. Pinto, J. Qi, B. Liu, H. Qiu, S.M. Du, H. Wang, W. Sun, Y. Wang, T.-J. Fu, Y. Zhang, J.E. Mueller and J. Chen, Nicks, Nodes, and New Motifs for DNA Nanotechnology, *Frontiers of Nano-Optoelectronic Systems*, ed. by L. Pavesi & E. Buzanova, Kluwer, Dordrecht, 177-198, (2000)

- [SRR76] N.C. Seeman, J.M. Rosenberg & A. Rich, Sequence Specific Recognition of Double Helical Nucleic Acids by Proteins, *Proc. Nat. Acad. Sci. (USA)* 73, 804-808, (1976)
- [SRS76] N.C. Seeman, J.M. Rosenberg, F.L. Suddath, J.-J.P. Kim & A. Rich, RNA Double Helical Fragments at Atomic Resolution I: The Crystal and Molecular Structure of Adenylyl-3',5'-uridine Hexahydrate. *J. Mol. Biol.* 104, 109-144, (1976)
- [SS04] G. Serrano & N.C. Seeman, Nanotecnologia basada en ADN, *Revista de Química* 19, 11-20, (2004), in Spanish.
- [SLM+00] R. Sha, F. Liu, D.P. Millar N.C. Seeman, Atomic Force Microscopy of Parallel DNA Branched Junction Lattices, *Chem. & Biol.* 7, 743-751, (2000)
- [SLM+00] *R. Sha, F. Liu, D.P. Millar, N.C. Seeman, Atomic Force Microscopy of Parallel DNA Branched Junction Arrays, *Chemistry & Biology* 7, 743-751, (2000)
- [SLS02] *R. Sha, F. Liu and N.C. Seeman, Atomic Force Measurement of the Interdomain Angle in Symmetric Holliday Junctions, *Biochemistry* 41, 5950-5955, (2002)
- [SBG+04] W. Shen, M. Bruist, S. Goodman & N.C. Seeman, A Nanomechanical Device for Measuring the Excess Binding Energy of Proteins that Distort DNA, *Angew. Chem. Int. Ed.* 43, 4750-4752 (2004); *Angew.Chem.* 116, 4854-4856, (2004)
- [SYW+04] Z. Shen, H. Yan, T. Wang and N.C. Seeman, Paranemic Crossover DNA: A Generalized Holliday Structure with Applications in Nanotechnology *J. Am. Chem. Soc.* 126, 1666-1674, (2004)
- [SS04] W.B. Sherman and N.C. Seeman, A Precisely Controlled DNA Bipedal Walking Device, *NanoLetters* 4, 1203-1207; Erratum, 4, 1801-1801, (2004)
- [SY01] Simmel, F. C., Yurke, B., Using DNA to construct and power a nanoactuator, *Physical Review E* 63: 2001, in print.
- [SW05] David Soloveichik and Erik Winfree. Complexity of Compact Proofreading for Self-Assembled Patterns, *DNA Computing* 11, (2005)
- [SW05] David Soloveichik and Erik Winfree. Complexity of Self-Assembled Shapes. *DNA Computers* 10, LNCS volume 3384:344-354, (2005)
- [SW05] David Soloveichik and Erik Winfree. The Computational Power of Benenson Automata. *Theoretical Computer Science* 244(2-3):279-297, (2005)
- [SZL00] M. Su, B. Zheng, and J. Liu, "A Scalable CVD Method for the Synthesis of Single Walled Carbon Nanotubes with High Catalyst Productivity," *Chemical Physics Letters*, 322, 321-326, (2000)
- [SYR05] Sudheer Sahu, Peng Yin and John H. Reif, A Self-Assembly Model of Time-Dependent Glue Strength, Eleventh International Meeting on DNA Based Computers (DNA11), London, Ontario (June, 2005), Springer-Verlag, New York, NY, edited by Alessandra Carbone and Niles Pierce, Lecture Notes for Computer Science (LNCS), (2006)
- [TKK92] Tsai, D.E., Kenan, D.J. and Keene, J.D. In vitro selection of an RNA epitope immunologically cross-reactive with a peptide. *Proc. Nat. Acad. Sci., U.S.A.* 89:8864-8868, (1992)
- [TYM00] Turberfield, A. J., Yurke, B., and Mills Jr., A. P., DNA hybridization catalysts and molecular tweezers, in *DNA Based Computers V*, Winfree, E., and Gifford, D. K., editors, American Mathematical Society, (2000), pages 171-182
- [U97] Urry, D.W., Physical chemistry of biological free energy transduction as demonstrated by elastic protein-based polymers. *J. Pys. Chem.B*, 101, 11007, (1997)

- [WDS96] H. Wang, R.J. Di Gate, & N.C. Seeman, An RNA Topoisomerase, *Proc. Nat. Acad. Sci. (USA)* 93, 9477-9482, (1996)
- [WDS93] H. Wang, S.M. Du and N.C. Seeman, Tight Single-Stranded DNA Knots, *J. Biomol. Struct. & Dyns.* 10, 853-863, (1993)
- [WB04] Erik Winfree and Renat Bekbolatov. Proofreading Tile Sets: Error Correction for Algorithmic Self-Assembly. *DNA Computers 9*, LNCS volume 2943:126-144, (2004)
- [WER00] Winfree, E., Eng, T., & Rozenberg, G., String Tile Models for DNA Computing by Self-Assembly, In Condon, A., & Rozenberg, G. (eds.), *Proceedings of the 6th Annual Meeting on DNA Based Computers, held in Leiden, June 13-17, (2000)*
- [Win96] Winfree, Erik. "On the computational power of DNA annealing and ligation." In Lipton and Baum, editors, *DNA Based Computers*, pages 199-221, Providence RI. American Mathematical Society, (1996)
- [Win98] Winfree, Erik. Simulations of Computing by Self-Assembly. In Kari et al., editors, *Proceedings of the 4th DIMACS Meeting on DNA Based Computers*, (1998)
- [Win98] Winfree, E., Whiplash PCR for O(1) Computing, In Kari, L., Rubin, H., & Wood, D.H., (eds.), *Proceedings of the 4th DIMACS Meeting on DNA Based Computers*, held at the University of Pennsylvania, (1998)
- [Win00] Erik Winfree. Algorithmic Self-Assembly of DNA: Theoretical Motivations and 2D Assembly Experiments. *Journal of Biomolecular Structure and Dynamics*, 11 (S2): 263-270, (2000)
- [Win01] Erik Winfree, Tony Eng, Grzegorz Rozenberg. String Tile Models for DNA Computing by Self-Assembly. *DNA Based Computers 6*, LNCS 2054, pp. 63-88, 2001.
- [Win03] Erik Winfree. A brief review of algorithmic self-assembly of DNA. *NAE's The Bridge*, 33(4):31-38, (2003)
- [Win05] Erik Winfree. Self-Healing Tile Sets, abstract, *FNANO 2005 conference proceedings*, (2005)
- [WWS98] E. Winfree, F. Liu, L. A. Wenzler, and N.C. Seeman, Design and Self-Assembly of Two-Dimensional DNA Crystals, *Nature* 394, 539-544, (1998)
- [WYS98] Winfree, E., Yang, X., & Seeman, N.C., Universal computation via self-assembly of DNA: Some theory and experiments, In Landweber, L.F. & Baum, E.B. (eds.), *DNA Based Computers II: DIMACS Workshop, June 10-12, 1996*, American Mathematical Society, Providence, RI, (1998)
- [XLR+02] S. Xiao, F. Liu, A. Rosen, J.F. Hainfeld, N.C. Seeman, K.M. Musier-Forsyth & R.A. Kiehl, Self-Assembly of Nanoparticle Arrays by DNA Scaffolding, *J. Nanoparticle Research* 4, 313-317, (2002)
- [YFL+03] Hao Yan, Liping Feng, Thomas H. LaBean, and John Reif, DNA Nanotubes, *Parallel Molecular Computation of Pair-Wise XOR Using DNA String Tile*, Ninth International Meeting on DNA Based Computers (DNA9), Madison, Wisconsin, June 2-4, 2003, (Edited by Junghuei Chen and John Reif), Abstract in *Lecture Notes in Computer Science*, Springer-Verlag, New York, (2003). Published as *Parallel Molecular Computations of Pairwise Exclusive-Or (XOR) Using DNA "String Tile" Self-Assembly* in *Journal of American Chemistry Society(JACS)*, Vol. 125, No. 47, pp. 14246-14247, (2003)
- [YLF+03] Hao Yan, Thomas H. LaBean, Liping Feng, and John H. Reif, Directed Nucleation Assembly of Barcode Patterned DNA Lattices, *Proceedings of the National Academy of*

Science(PNAS), Volume 100, No. 14, pp. 8103-8108, (2003)

[YPF+03] Hao Yan, Sung Ha Park, Liping Feng, John Reif, and Thomas H. LaBean, *4x4 DNA Tile and Lattices: Characterization, Self-Assembly and Metallization of a Novel DNA Nanostructure Motif*, Ninth International Meeting on DNA Based Computers (DNA9), Madison, Wisconsin, June 2-4, 2003, (Edited by Junghuei Chen and John Reif), Lecture Notes in Computer Science, Springer-Verlag, New York, (2003). Published as Hao Yan, Sung Ha Park, Gleb Finkelstein, John H. Reif, and Thomas H. LaBean, DNA-Templated Self-Assembly of Protein Arrays and Highly Conductive Nanowires, *Science*, Vol. 301, pp. 1882-1884, (2003)

[YS03] H. Yan and N.C. Seeman, Edge-Sharing Motifs in DNA Nanotechnology. *Journal of Supramolecular Chemistry* 1, 229-237, (2003)

[YZS+02] H. Yan, X. Zhang, Z. Shen and N.C. Seeman, A Robust DNA Mechanical Device Controlled by Hybridization Topology, *Nature* 415, 62-65, (2002)

[YGB+05] Peng Yin, Bo Guo, Christina Belmore, Will Palmeri, Erik Winfree, Thomas H. LaBean, John H. Reif, TileSoft: Sequence Optimization Software For Designing DNA Secondary Structures, Poster, Tenth International Meeting on DNA Based Computers (DNA10), Milano, Italy, June 7-10, 2004. Lecture Notes in Computer Science (Edited by C Ferretti, G. Mauri and C. Zandron), Vol. 3384, Springer-Verlag, New York, (2005)

[YTR05] Peng Yin, Andrew J. Turberfield, John H. Reif, Design of an Autonomous DNA Nanomechanical Device Capable of Universal Computation and Universal Translational Motion, Tenth International Meeting on DNA Based Computers (DNA10), (Edited by C Ferretti, G. Mauri and C. Zandron), Milano, Italy, June 7-10, 2004. Lecture Notes in Computer Science(LNCS), Number 3384, Springer-Verlag, New York, pp 426-444, (2005)

YTR05] Peng Yin, Andrew J. Turberfield, John H. Reif, Design of Autonomous DNA Cellular Automata, Eleventh International Meeting on DNA Based Computers (DNA11), London, Ontario (June, 2005), Springer-Verlag, New York, NY, edited by Alessandra Carbone and Niles Pierce, Lecture Notes for Computer Science (LNCS), (2006)

[YTS+05] Peng Yin, Andrew J. Turberfield, Sudheer Sahu, John H. Reif, Designs for Autonomous Unidirectional Walking DNA Devices, Tenth International Meeting on DNA Based Computers (DNA10), Milano, Italy, June 7-10, 2004. Lecture Notes in Computer Science (Edited by C Ferretti, G. Mauri and C. Zandron), Vol. 3384, Springer-Verlag, New York, pp 410-425, (2005)

[YYG+04] Peng Yin, Hao Yan, Xiaoju G. Daniel, Andrew J. Turberfield, John H. Reif, A Unidirectional DNA Walker Moving Autonomously Along a Linear Track, *Angewandte Chemie [International Edition]*, Volume 43, Number 37, pp 4906-4911, (2004)

[YMC99] Yurke, B., Mills, Jr., A. P., and Cheng, S. L. DNA implementation of addition in which the input strands are separate from the operator strands, *Biosystems* 52: 165-174, 1999.

[YTM+00] B. Yurke, A.J. Turberfield, A.P. Mills, Jr., F.C. Simmel & J.E. Neumann, A DNA-Fuelled Molecular Machine Made of DNA, *Nature* 406, 605-608, (2000)

[YZS+01] H. Yan, X. Zhang, Z. Shen & N.C. Seeman, A Robust Rotary Sequence-Dependent DNA Nanomechanical Device, in preparation.

[YWQ+98] X. Yang, L.A. Wenzler, J. Qi, X. Li and N.C. Seeman, Ligation of DNA Triangles Containing Double Crossover Molecules, *J. Am. Chem. Soc.* 120, 9779-9786, (1998)

- [ZPG+01] D. Zanchet, C. M. M., Wolfgang J. Parak, Daniele Gerion, and A. Paul Alivisatos, "Electrophoretic Isolation of Discrete Au Nanocrystal/DNA Conjugates," *Nano Letters*, 1, 32-35, (2001)
- [ZS94] Y. Zhang and N.C. Seeman, The Construction of a DNA Truncated Octahedron, *J. Am. Chem. Soc.* 116, 1661-1669, (1994)
- [ZYS+02] X. Zhang, H. Yan, Z. Shen and N.C. Seeman, Paranemic Cohesion of Topologically-Closed DNA Molecules, *J Am. Chem. Soc.* 124, 12940-12941, (2002)
- [ZLC+03] L. Zhu, P.S. Lukeman, J. Canary & N.C. Seeman, Nylon/DNA: Single-Stranded DNA with Covalently Stitched Nylon Lining, *J. Am. Chem. Soc.* 125, 10178-10179, (2003)

Appendix A: Papers Resulting from this Contract

REFERENCES

PAPERS BY REIF ON RESEARCH SUPPORTED BY THIS CONTRACT

1. T. H. LaBean, Yan, H., Kopatsch, J., Liu, F., Winfree, E., Reif, J.H. and Seeman, N.C., The construction, analysis, ligation and self-assembly of DNA triple crossover complexes, *Journal of American Chemistry Society (JACS)* 122, 1848-1860, (2000)
2. A. Gehani, T. H. LaBean, and J.H. Reif, DNA-based Cryptography, *Proc. DNA Based Computers V: Cambridge, MA, June 14-16, 1999*. Published in DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Volume 54, edited by E. Winfree and D.K. Gifford, American Mathematical Society, Providence, RI, pp. 233-249, (2000). [PDF] talk slides: [PDF]. Published as an invited chapter in "Aspects of Molecular Computing - Essays dedicated to Tom Head on the occasion of his 70th Birthday", Springer Verlag series in Natural Computing (edited by N. Jonoska, G. Paun and G. Rozenberg) LNCS 2950 Festschrift, Springer, pp. 167-188, (2004)
3. T. H. LaBean, E. Winfree, and J.H. Reif, Experimental Progress in Computation by Self-Assembly of DNA Tilings, Published in DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Volume 54, edited by E. Winfree and D.K. Gifford, American Mathematical Society, Providence, RI, pp. 123-140, (2000)
4. J.H. Reif and T. H. LaBean, and N.C. Seeman. Programmable Assembly at the Molecular Scale: Self-Assembly of DNA Lattices, Invited paper, 2001 IEEE International Conference on Robotics and Automation (ICRA2001), Seoul, Korea, ed. Lee Beom (May, 2001). [PDF]
5. J.H. Reif and T. H. LaBean, Computationally Inspired Biotechnologies: Improved DNA Synthesis and Associative Search Using Error-Correcting Codes and Vector-Quantization, Sixth International Meeting on DNA Based Computers (DNA6), DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Edited by A. Condon and G. Rozenberg. Lecture Notes in Computer Science, Springer-Verlag, Berlin Heidelberg, vol. 2054, pp. 145-172, (2001)
6. J.H. Reif, T.H. LaBean, and N.C. Seeman, Challenges and Applications for Self-Assembled DNA Nanostructures, *Proc. Sixth International Workshop on DNA-Based Computers*, Leiden, The Netherlands, June, 2000. DIMACS Series in Discrete Mathematics and Theoretical Computer Science, Edited by A. Condon and G. Rozenberg. Lecture Notes in Computer Science, Springer-Verlag, Berlin Heidelberg, vol. 2054, pp. 173-198, (2001)
7. C. Mao, LaBean, T.H. Reif, J.H., Seeman, Logical Computation Using Algorithmic Self-Assembly of DNA Triple-Crossover Molecules, *Nature*, vol. 407, Sept. 28 2000, pp. 493-495; C. Erratum: *Nature* 408, 750-750, (2000)

8. J. H. Reif, T. H. LaBean, M. Pirrung, V. Rana, B. Guo, K. Kingsford, and G. Wickham, Experimental Construction of Very Large Scale DNA Databases with Associative Search Capability, Seventh International Meeting on DNA Based Computers (DNA7), Tampa, FL, June 11-13, 2001. Lecture Notes in Computer Science, Springer-Verlag, New York, Volume 2340, pages 231-247, (2002). Also published as John H. Reif, Michael Hauser, Michael Pirrung, and Thomas LaBean, Application of Biomolecular Computing to Medical Science: A Biomedical Database System for Storage and Retrieval of Genetic Information and Material, Chapter 3 of Complex Systems Science in Biomedicine (Edited by Tom Deisboeck), Kluwer-Springer Publishers, Chapter 3, pages 701-735, (2005)
9. J. H. Reif, DNA Lattices: A Method for Molecular Scale Patterning and Computation, special issue on Bio-Computation, Computer and Scientific Engineering Magazine, IEEE Computer Society, Vol. 4, No. 1, pp 32-41, (2002)
10. J. H. Reif, The Emerging Discipline of Biomolecular Computation in the US, invited paper to the special issue on Biomolecular Computing, New Generation Computing, edited by Masami Hagiya, Masayuki Yamamura, and Tom Head, Vol. 20, No. 3, pp. 217-236, (2002)
11. J. H. Reif, Molecular Assembly and Computation: From Theory to Experimental Demonstrations, plenary paper, Twenty Ninth International Colloquium on Automata, Languages, and Programming(ICALP), Málaga, Spain (July 8, 2002). Lecture Notes in Computer Science, New York, Volume 2380, pages 1-21, (2002)
12. J. H. Reif, The Design of Autonomous DNA Nanomechanical Devices: Walking and Rolling DNA. DNA Based Computers (DNA8), Sapporo, Japan, June 10-13, 2002, (Edited by Masami Hagiya and Azuma Ohuchi), Lecture Notes in Computer Science, No. 2568, Springer-Verlag, New York, (2003), pages 22-37. Published in Natural Computing, DNA8 special issue, Vol. 2, p 439-461, (2003)
13. Dage Liu, John H. Reif, Thomas H. LaBean, DNA Nanotubes, Construction and Characterization of Filaments Composed of TX-tile Lattice. DNA Based Computers (DNA8), Sapporo, Japan, June 10-13, 2002, (Edited by Masami Hagiya and Azuma Ohuchi), Lecture Notes in Computer Science, No. 2568, Springer-Verlag, New York, (2003), pages 10-21. Published as DNA nanotubes self-assembled from triple-crossover tiles as templates for conductive nanowires, Proceedings of the National Academy of Science(PNAS), Vol. 101, No. 3, pp 717-722, (2004)
14. John H. Reif, DNA Computation - Perspectives: Successes and Challenges, Science, 296: 478-479, (2002)
15. Hao Yan, Thomas H. LaBean, Liping Feng, and John H. Reif, Directed Nucleation Assembly of Barcode Patterned DNA Lattices, Proceedings of the National Academy of Science(PNAS), Volume 100, No. 14, pp. 8103-8108, (2003)
16. Hao Yan, Liping Feng, Thomas H. LaBean, and John Reif, DNA Nanotubes, *Parallel Molecular Computation of Pair-Wise XOR Using DNA String Tile*, Ninth International

- Meeting on DNA Based Computers (DNA9), Madison, Wisconsin, June 2-4, 2003, (Edited by Junghuei Chen and John Reif), Abstract in Lecture Notes in Computer Science, Springer-Verlag, New York, (2003). Published as Parallel Molecular Computations of Pairwise Exclusive-Or (XOR) Using DNA "String Tile" Self-Assembly in Journal of American Chemistry Society(JACS), Vol. 125, No. 47, pp. 14246-14247, (2003)
17. Hao Yan, Sung Ha Park, Liping Feng, John Reif, and Thomas H. LaBean, *4x4 DNA Tile and Lattices: Characterization, Self-Assembly and Metallization of a Novel DNA Nanostructure Motif*, Ninth International Meeting on DNA Based Computers (DNA9), Madison, Wisconsin, June 2-4, 2003, (Edited by Junghuei Chen and John Reif), Lecture Notes in Computer Science, Springer-Verlag, New York, (2003). Published as Hao Yan, Sung Ha Park, Gleb Finkelstein, John H. Reif, and Thomas H. LaBean, DNA-Templated Self-Assembly of Protein Arrays and Highly Conductive Nanowires, Science, Vol. 301, pp. 1882-1884, (2003)
 18. Liping Feng, Sung Ha Park, John H. Reif, and Hao Yan, A Two-State DNA Lattice Switched by DNA Nanoactuator, Angewandte Chemie [International Edition], Vol. 42, pp. 4342-4346, (2003)
 19. Li, H.; Park, S. H.; Reif, John H.; LaBean, T. H.; Yan, H., DNA-Templated Self-Assembly of Protein and Nanoparticle Linear Arrays, Journal of American Chemistry Society(JACS), Vol. 126, No. 2, pp. 418-419, (2004)
 20. Thomas H. LaBean, Hao Yan, Sung Ha Park, Liping Feng, Peng Yin, Hanying Li, Sang Jung Ahn, Dage Liu, Xiaoju Guan, and John H. Reif, Overview of New Structures for DNA-Based Nanofabrication and Computation, Invited paper to appear in Information Sciences, (2005)
 21. John H. Reif, Sudheer Sahu, Peng Yin, Compact Error-Resilient Computational DNA Tiling Assemblies, Tenth International Meeting on DNA Based Computers (DNA10), Milano, Italy, June 7-10, 2004. Lecture Notes in Computer Science (Edited by C Ferretti, G. Mauri and C. Zandron), Vol. 3384, Springer-Verlag, New York, (2005), pp 293-307. Published as an invited chapter in "Aspects of Molecular Computing - Essays dedicated to Nadrian Seeman on the occasion of his 60th Birthday", Springer Verlag series in Natural Computing (edited by Natasha Jonoska) Lecture Notes in Computer Science(LNCS) Festschrift, (2006)
 22. Peng Yin, Andrew J. Turberfield, Sudheer Sahu, John H. Reif, Designs for Autonomous Unidirectional Walking DNA Devices, Tenth International Meeting on DNA Based Computers (DNA10), Milano, Italy, June 7-10, 2004. Lecture Notes in Computer Science (Edited by C Ferretti, G. Mauri and C. Zandron), Vol. 3384, Springer-Verlag, New York, pp 410-425, (2005)
 23. Peng Yin, Hao Yan, Xiaoju G. Daniel, Andrew J. Turberfield, John H. Reif, A Unidirectional DNA Walker Moving Autonomously Along a Linear Track, Angewandte Chemie [International Edition], Volume 43, Number 37, pp 4906-4911, (2004)
 24. Peng Yin, Andrew J. Turberfield, John H. Reif, Design of an Autonomous DNA Nanomechanical Device Capable of Universal Computation and Universal Translational

- Motion, Tenth International Meeting on DNA Based Computers (DNA10), (Edited by C Ferretti, G. Mauri and C. Zandron), Milano, Italy, June 7-10, 2004. Lecture Notes in Computer Science(LNCS), Number 3384, Springer-Verlag, New York, pp 426-444, (2005)
25. Peng Yin, Bo Guo, Christina Belmore, Will Palmeri, Erik Winfree, Thomas H. LaBean, John H. Reif, TileSoft: Sequence Optimization Software For Designing DNA Secondary Structures, Poster, Tenth International Meeting on DNA Based Computers (DNA10), Milano, Italy, June 7-10, 2004. Lecture Notes in Computer Science (Edited by C Ferretti, G. Mauri and C. Zandron), Vol. 3384, Springer-Verlag, New York, (2005)
 26. John H. Reif, DNA-Based Nano-Engineering: DNA and its Enzymes as the Engines of Creation at the Molecular Scale, Invited Talk, Tenth International Meeting on DNA Based Computers (DNA10), Milano, Italy, June 7-10, 2004. Lecture Notes in Computer Science (Edited by C Ferretti, G. Mauri and C. Zandron), Vol. 3384, Springer-Verlag, New York, (2005)
 27. John H. Reif, Thomas LaBean, Sudheer Sahu, Hao Yan, Peng Yin, Design, Simulation, and Experimental Demonstration of Self-Assembled DNA Nanostructures and DNA Motors, Unconventional Programming Paradigms: International Workshop UPP 2004, Le Mont Saint Michel, France, September 15-17, 2004. Published by Springer-Verlag Berlin Heidelberg, edited by Jean-Pierre Banâtre, Pascal Fradet, Jean-Louis Giavitto, Olivier Michel, Lecture Notes for Computer Science (LNCS) 3566, pp 166-180, (2005)
 28. Sung Ha Park, Hao Yan, John H Reif, Thomas H LaBean, and Gleb Finkelstein, Electronic nanostructures templated on self-assembled DNA scaffolds, *Nanotechnology* 15, p. S525–S527, (2004)
 29. Sung Ha Park, Peng Yin, John Reif, Thomas LaBean, and Hao Yan, Programmable DNA Self-assemblies for Nanoscale Organization of Ligands and Proteins, *Nano Letters* (Communication), Volume 5, Number 4, pp 729-733, (2005)
 30. Sung Ha Park, Robert Barish, John Reif, Gleb Finkelstein, Hao Yan and Thomas LaBean, Three-Helix Bundle DNA Tiles Self-Assemble into 2D Lattice or 1D Templates for Silver Nanowires, *Nano Letters* (Communication), Volume 5, Number 4, pp. 693-696 (2005)
 31. Sung Ha Park, John Reif, Thomas LaBean and Gleb Finkelstein, Metallic Nanowires Templated on Native and Synthetic double-stranded DNA Scaffolds, Submitted for publication, (2005)
 32. Sung Ha Park, Constantin Pistol, Sang Jung Ahn, John H. Reif, Alvin R. Lebeck, Chris Dwyer, and Thomas H. LaBean, Finite-Size, Fully Addressable DNA Tile Lattices Formed by Hierarchical Assembly Procedures, accepted to appear in *Angewandte Chemie* [International Edition] (2005)
 33. John H. Reif, Sudheer Sahu, and Peng Yin, Complexity of Graph Self-Assembly in Accretive Systems and Self-Destructible Systems, Eleventh International Meeting on DNA Based

Computers (DNA11), London, Ontario (June, 2005), Springer-Verlag, New York, NY, edited by Alessandra Carbone and Niles Pierce, Lecture Notes for Computer Science (LNCS), (2006), Submitted for publication, (2005)

34. Peng Yin, Andrew J. Turberfield, John H. Reif, Design of Autonomous DNA Cellular Automata, Eleventh International Meeting on DNA Based Computers (DNA11), London, Ontario (June, 2005), Springer-Verlag, New York, NY, edited by Alessandra Carbone and Niles Pierce, Lecture Notes for Computer Science (LNCS), (2006), Submitted for publication, (2005)
35. Sudheer Sahu, Peng Yin and John H. Reif, A Self-Assembly Model of Time-Dependent Glue Strength, Eleventh International Meeting on DNA Based Computers (DNA11), London, Ontario (June, 2005), Springer-Verlag, New York, NY, edited by Alessandra Carbone and Niles Pierce, Lecture Notes for Computer Science (LNCS), (2006), Submitted for publication, (2005)
36. Sung Ha Park, Hanying Li, Robert Barish, John Reif, Gleb Finkelstein, Hao Yan and Thomas LaBean, Self-assembled DNA nanobundles as Templated for Silver Nanowires, Eleventh International Meeting on DNA Based Computers (DNA11), London, Ontario (June, 2005), Springer-Verlag, New York, NY, edited by Alessandra Carbone and Niles Pierce, Lecture Notes for Computer Science (LNCS), (2006), Submitted for publication, (2005)
37. Sung Ha Park, John Reif, Thomas LaBean and Gleb Finkelstein, Metallic Nanowires Templated on Native and Synthetic double-stranded DNA Scaffolds, Eleventh International Meeting on DNA Based Computers (DNA11), London, Ontario (June, 2005), Springer-Verlag, New York, NY, edited by Alessandra Carbone and Niles Pierce, Lecture Notes for Computer Science (LNCS), (2006), Submitted for publication, (2005)

PAPERS BY SEEMAN ON RESEARCH SUPPORTED BY THIS CONTRACT

1. T. LaBean, H. Yan, J. Kopatsch, F. Liu, E. Winfree, J.H. Reif and N.C. Seeman, The Construction of DNA Triple Crossover Molecules, *Journal of the American Chemical Society* 122, 1848-1860, (2000)
2. N.C. Seeman, F. Liu, C. Mao, X. Yang, L. A. Wenzler, R. Sha, W. Sun, Z. Shen, X. Li, J. Qi, Y. Zhang, T.-J. Fu, J. Chen and E. Winfree, Two Dimensions and Two States in DNA Nanotechnology, *Proceedings of the 11th Conversation in Biomolecular Stereodynamics*, ed. by R.H. Sarma and M.H. Sarma, Adenine Press, New York, 253-262, (2000)
3. N.C. Seeman, DNA Nanotechnology: From Topological Control to Structural Control, in *Pattern Formation in Biology, Vision and Dynamics*, ed. by A.Carbone, M.Gromov, P.Pruzinkiewicz, World Scientific Publishing Company, Singapore, 271-309, (2000)

4. N.C. Seeman, C. Mao, F. Liu, R. Sha, X. Yang, L. Wenzler, X. Li, Z. Shen, H. Yan, P. Sa-Ardyen, X. Zhang, W. Shen, J. Birac, P. Lukeman, Y. Pinto, J. Qi, B. Liu, H. Qiu, S.M. Du, H. Wang, W. Sun, Y. Wang, T.-J. Fu, Y. Zhang, J.E. Mueller and J. Chen, Nicks, Nodes, and New Motifs for DNA Nanotechnology, *Frontiers of Nano-Optoelectronic Systems*, ed. by L. Pavesi & E. Buzanova, Kluwer, Dordrecht, 177-198, (2000)
5. *R. Sha, F. Liu, D.P. Millar N.C. Seeman, Atomic Force Microscopy of Parallel DNA Branched Junction Arrays, *Chemistry & Biology* 7, 743-751, (2000)
6. C. Mao, T. LaBean, J.H. Reif and N.C. Seeman, Logical Computation Using Algorithmic Self-Assembly of DNA Triple Crossover Molecules, *Nature* 407, 493-496, (2000)
7. N.C. Seeman, In the Nick of Space: Generalized Nucleic Acid Complementarity and the Development of DNA Nanotechnology, *Synlett* 2000, 1536-1548, (2000)
8. N.C. Seeman, DNA Nicks and Nodes and Nanotechnology, *NanoLetters* 1, 22-26, (2001)
9. J.H. Reif, T.H. LaBean & N.C. Seeman, Challenges and Applications for Self-Assembled DNA Nanostructures, Sixth International Workshop on DNA-Based Computers, DNA 2000, Leiden, The Netherlands, (June, 2000) ed. A. Condon, G. Rozenberg. Springer-Verlag, Berlin Heidelberg, Lecture Notes in Computer Science 2054, 173-198, (2001)
10. H. Yan, X. Zhang, Z. Shen and N.C. Seeman, A Robust DNA Mechanical Device Controlled by Hybridization Topology, *Nature* 415, 62-65, (2002)
11. N.C. Seeman, DNA Nanotechnology: Life's Central Performer in a New Role, *Biological Physics Newsletter* 2 (1) 2-6, (2002)
12. N.C. Seeman, It Started with Watson and Crick, But it Sure Didn't End There: Pitfalls and Possibilities beyond the Classic Double Helix, *Natural Computing* 1, 53-84, (2002)
13. N.C. Seeman & A.M. Belcher, Emulating Biology: Nanotechnology from the Bottom Up, *Proceedings of the National Academy of Sciences (USA)* 99 (supp. 2), 6451-6455, (2002)
14. *R. Sha, F. Liu and N.C. Seeman, Atomic Force Measurement of the Interdomain Angle in Symmetric Holliday Junctions, *Biochemistry* 41, 5950-5955, (2002)
15. N.C. Seeman, Key Experimental Approaches in DNA Nanotechnology, *Current Protocols in Nucleic Acid Chemistry*, Unit 12.1, John Wiley & Sons, New York, (2002)
16. A. Carbone and N.C. Seeman, Circuits and Programmable Self-Assembling DNA Structures, *Proc. Nat. Acad. Sci. (USA)* 99 12577-12582, (2002)

17. X. Zhang, H. Yan, Z. Shen and N.C. Seeman, Paranemic Cohesion of Topologically-Closed DNA Molecules, *J Am. Chem. Soc.* 124, 12940-12941, (2002)
18. S. Xiao, F. Liu, A. Rosen, J.F. Hainfeld, N.C. Seeman, K.M. Musier-Forsyth & R.A. Kiehl, Self-Assembly of Nanoparticle Arrays by DNA Scaffolding, *J. Nanoparticle Research* 4, 313-317, (2002)
19. A. Carbone and N.C. Seeman, Fractal Designs Based on DNA Parallelogram Structures, *Natural Computing* 1, 469-480, (2002)
20. N.C. Seeman, DNA in a Material World, *Nature* 421, 33-37, (2003)
21. N.C. Seeman, Structural DNA Nanotechnology: A New Organizing Principle for Advanced Nanomaterials, *Materials Today* 6 (7), 24-29, (2003)
22. P. Sa-Ardyen, N. Jonoska and N.C. Seeman, Self-Assembling DNA Graphs, *DNA-Based Computers VIII*, LNCS 2568, Springer-Verlag, Berlin, 1-9, (2003)
23. H. Yan and N.C. Seeman, Edge-Sharing Motifs in DNA Nanotechnology. *Journal of Supramolecular Chemistry* 1, 229-237, (2003)
24. *P. Sa-Ardyen, A.V. Vologodskii and N.C. Seeman, The Flexibility of DNA Double Crossover Molecules. *Biophysical Journal* 84, 3829-3837, (2003)
25. N. Jonoska, P. Sa-Ardyen and N.C. Seeman, Computation by Self-Assembly of DNA Graphs, *Journal of Genetic Programming and Evolvable Machines* 4, 123-137, (2003)
26. N.C. Seeman, Biochemistry and Structural DNA Nanotechnology: An Evolving Symbiotic Relationship, *Biochemistry* 42, 7259-7269, (2003)
27. L. Zhu, P.S. Lukeman, J. Canary & N.C. Seeman, Nylon/DNA: Single-Stranded DNA with Covalently Stitched Nylon Lining, *J. Am. Chem. Soc.* 125, 10178-10179, (2003)
28. Carbone and N.C. Seeman, Coding and Geometrical Shapes in Nanostructures: a Fractal DNA-Assembly, *Natural Computing* 2, 133-151, (2003)
29. N.C. Seeman, DNA Nanostructures for Mechanics and Computing: Nonlinear Thinking With Life's Central Molecule. *NanoBiotechnology*. Editors, Chad Mirkin and Christof Niemeyer, Wiley-VCH Verlag GmbH & Co., KgaA, Weinheim, Chapter 20, pp 308-318, (2004)
30. N.C. Seeman, DNA: Beyond the Double Helix, *Macromolecular Symposia* 201, 237-244, (2003)

31. N.C. Seeman, At the Crossroads of Chemistry, Biology and Materials: Structural DNA Nanotechnology, *Chemistry & Biology* 10, 1151-1159, (2003)
32. P. Sa-Ardyen, N. Jonoska and N.C. Seeman, Self-Assembling DNA Graphs, *Natural Computing*, 2, 427-438, (2003)
33. N.C. Seeman, DNA Nanostructures for Mechanics and Computing: Nonlinear Thinking With Life's Central Molecule. *NanoBiotechnology*. Editors, Chad Mirkin and Christof Niemeyer, Wiley-VCH Verlag GmbH & Co., Weinheim, Chapter 20, pp 308-318, (2004)
34. Z. Shen, H. Yan, T. Wang and N.C. Seeman, Paranemic Crossover DNA: A Generalized Holliday Structure with Applications in Nanotechnology *J. Am. Chem. Soc.* 126, 1666-1674, (2004)
35. N. Jonoska, S. Liao, & N.C. Seeman, Transducers with programmable input by DNA Self-assembly, *Aspects of Molecular Computing, Lecture Notes in Computer Science* 2340, Springer-Verlag, Berlin, 219-240, (2004)
36. A. Carbone & N.C. Seeman, Molecular Tiling and DNA Self-Assembly, *Aspects of Molecular Computing, Lecture Notes in Computer Science* 2340, Springer-Verlag, Berlin, 61-83, (2004)
37. N.C. Seeman. Nanotechnology and the Double Helix, *Scientific American.*, 290, (6) 64-75, (2004)
38. P. Sa-Ardyen, N. Jonoska and N.C. Seeman, The Construction of Graphs Whose Edges are DNA Helix Axes, *J. Am. Chem. Soc.*, 126, 6648-6657, (2004)
39. W.B. Sherman and N.C. Seeman, A Precisely Controlled DNA Bipedal Walking Device, *NanoLetters* 4, 1203-1207, Erratum, 4, 1801-1801, (2004)
40. P.S. Lukeman, A. Mittal, & N.C. Seeman, Two Dimensional PNA/DNA Arrays: Estimating the Helicity of Unusual Nucleic Acid Polymers, *Chemical Communications* 2004, 1694-1695, (2004)
41. N.C. Seeman, DNA Nanotechnology, *Encyclopedia of Supramolecular Chemistry*, Marcel Dekker, New York, 475-483, (2004)
42. A. Carbone, C. Mao, P. Constantinou, B. Ding, J. Kopatsch, W. B. Sherman and N.C. Seeman, 3D Fractal DNA Assembly from Coding, Geometry and Protection, *Natural Computing* 3, 235-252, (2004)

43. A. Ding, R. Sha & N.C. Seeman, Pseudohexagonal 2D DNA Crystals from Double Crossover Cohesion, *J. Am. Chem. Soc.* 126, 10230-10231, (2004)
44. W. Shen, M. Bruist, S. Goodman & N.C. Seeman, A Nanomechanical Device for Measuring the Excess Binding Energy of Proteins that Distort DNA, *Angew. Chem. Int. Ed.* 43, 4750-4752 (2004); *Angew.Chem.* 116, 4854-4856, (2004)
45. S. Liao and N.C. Seeman, Translation of DNA Signals into Polymer Assembly Instructions, *Science* 306, 2072-2074, (2004)
46. J. D. Le, Y. Pinto, N.C. Seeman, K. Musier-Forsyth, T. A. Taton and R.A. Kiehl, Self-Assembly of Nanoelectronic Component Arrays by In Situ Hybridization to 2D DNA Scaffolding, *NanoLetters* 4, 2343-2347, (2004)
47. G. Serrano & N.C. Seeman, Nanotecnologia basada en ADN, *Revista de Química* 19, 11-20, (2004), in Spanish.
48. N.C. Seeman and P.S. Lukeman, Nucleic Acid Nanostructures, *Reports on Progress in Physics* 68, 237-270, (2005)
49. N.C. Seeman, B. Ding , S. Liao , T. Wang, W.B. Sherman, P.E. Constantinou, J. Kopatsch, C. Mao, R. Sha, F. Liu, H. Yan & P.S. Lukeman, Experiments in Structural DNA Nanotechnology: Arrays and Devices , *Proc. SPIE; Nanofabrication: Technologies, Devices and Applications* 5592, 71-81, (2005)
50. N.C. Seeman, From Genes to Machines: DNA Nanomechanical Devices, *Trends in Biochemical Sciences* 30, 119-235, (2005)
51. N.C. Seeman. Structural DNA Nanotechnology: An Overview. *Methods in Molecular Biology* 303: Bionanotechnology Protocols, Editors, Sandra J. Rosenthal and David W. Wright, Humana Press, Totowa, NJ, pp. 143-166, (2005)
52. F. Mathieu, S. Liao, C. Mao, J. Kopatsch, T. Wang, and N.C. Seeman, Six-Helix Bundles Designed from DNA, *NanoLetters* 5, 661-665, (2005)
53. Sha, R., Zhang, X., Liao, S., Constantinou, P.E., Ding, B., Wang, T., Garibotti, A.V., Zhong, H., Israel, L.B., Wang, X., Wu, G., Chakraborty, B., Chen, J., Zhang, Y., Mao, C., Yan, H., Kopatsch, J., Zheng, J., Lukeman, P.S., Sherman, W.B. and Seeman, N.C., Motifs and Methods in Structural DNA Nanotechnology, *Proc. Intl. Conf. Nanomaterials, NANO 2005*, July 13-15, 2005, Mepco Schlenk Engineering College, Srivakasi, India, V. Rajendran, ed., pp. 3-10, (2005)

PAPERS BY WINFREE ON RESEARCH SUPPORTED BY THIS CONTRACT

1. Thomas H. LaBean, Hao Yan, Jens Kopatsch, Furong Liu, Erik Winfree, John H. Reif, Nadrian C. Seeman. Construction, analysis, ligation, and self-assembly of DNA triple crossover complexes, in *Journal of the American Chemical Society*, 122(9): 1848-1860, (2000)
2. Erik Winfree. Algorithmic Self-Assembly of DNA: Theoretical Motivations and 2D Assembly Experiments. *Journal of Biomolecular Structure and Dynamics*, 11 (S2): 263-270, (2000)
3. Nadrian C. Seeman, Furong Liu, Chengde Mao, Xiaoping Yang, Lisa A. Wenzler, Ruojie Sha, Weiqiong Sun, Zhiyong Shen, Xiaojun Li, Jing Qi, Yuwen Zhang, Tsu-Ju Fu, Junghuei Chen and Erik Winfree, Two Dimensional and Two States in DNA Nanotechnology. *Journal of Biomolecular Structure and Dynamics*, 11 (S2): 253-262, (2000)
4. Erik Winfree, Tony Eng, Grzegorz Rozenberg. String Tile Models for DNA Computing by Self-Assembly. *DNA Based Computers 6*, LNCS 2054, pp. 63-88, (2001)
5. Niles Pierce and Erik Winfree. Protein Design is NP-Hard. *Protein Engineering*, v15, pp. 779-782, (2002)
6. Erik Winfree. A brief review of algorithmic self-assembly of DNA. *NAE's The Bridge*, 33(4):31-38, (2003)
7. Paul W.K. Rothemund, Nick Papadakis, Erik Winfree. Algorithmic Self-Assembly of DNA Sierpinski Triangles. *PLoS Biology* 2 (12) p424, (2004)
8. Paul W.K. Rothemund, Axel Ekani-Nkodo, Nick Papadakis, Ashish Kumar, Deborah Kuchnir Fygenson, Erik Winfree. Design and Characterization of Programmable DNA Nanotubes, *JACS* 126(50):16344-16353, (2004)
9. David Soloveichik and Erik Winfree. Complexity of Self-Assembled Shapes. *DNA Computers 10*, LNCS volume 3384:344-354, (2005)
10. Robert M. Dirks, Milo Lin, Erik Winfree and Niles A. Pierce. Paradigms for Computational Nucleic Acid Design. *Nucleic Acids Research*: 32(4):1392-1403, (2004)
11. Erik Winfree and Renat Bekbolatov. Proofreading Tile Sets: Error Correction for Algorithmic Self-Assembly. *DNA Computers 9*, LNCS volume 2943:126-144, (2004)
12. Matthew Cook, Paul W.K. Rothemund, and Erik Winfree. Self-Assembled Circuit Patterns. *DNA Computers 9*, LNCS volume 2943:91-107, (2004)

13. Rebecca Schulman, Shaun Lee, Nick Papadakis, and Erik Winfree. One Dimensional Boundaries for DNA Tile Self-Assembly. DNA Computers 9, LNCS volume 2943:108-125, (2004)
14. Rob D. Barish, Paul W. K. Rothemund, and Erik Winfree, Two Computational Primitives for Algorithmic Self-Assembly: Copying and Counting, , to appear 2005.
15. David Soloveichik and Erik Winfree. Complexity of Compact Proofreading for Self-Assembled Patterns, DNA Computing 11, (2005)
16. Erik Winfree. Self-Healing Tile Sets, abstract, FNANO 2005 conference proceedings, 2005.
17. Rebecca Schulman and Erik Winfree. Self-Replication and Evolution of DNA Crystals, ECAL 2005, in LNCS 3630:734-743, (2005)
18. David Soloveichik and Erik Winfree. The Computational Power of Benenson Automata. Theoretical Computer Science 244(2-3):279-297, (2005)
19. Georg Seelig and Bernard Yurke and Erik Winfree. DNA Hybridization Catalysts and Catalyst Circuits. DNA Computers 10, LNCS volume 3384:329-343, (2005)
20. Rebecca Schulman and Erik Winfree. Programmable Control of Nucleation for Algorithmic Self-Assembly. DNA Computers 10, LNCS volume 3384:319-328, (2005)

Appendix B: Comparison with other Research in NanoAssembly

Comparison with other Methods for Nanoscale Constructs.

The overarching goal of this research contract was to make general purpose assembly of many complicated nanoscale constructs more practical and routine. Other approaches towards this broad goal have been proposed, but they tend to have drawbacks that are not easily overcome.

Overview of Other Approaches for Nanoscale Constructs:

B1.1 Lithography: Conventional top-down assembly methods do not extend to the molecular scale. In particular, lithography, the most successful micro-miniaturization technique so far, will not scale down to the 10nm regime. There is a general consensus that extending lithography to size scales smaller than 50nm will be extraordinarily difficult, if it will work at all. This is still more than one order of magnitude away from atomic precision, which is achieved by biological organisms. Going to short wavelength x-rays or e-beams could in principle achieve nm resolution, but the energy imparted by such hard radiation will destroy sensitive molecular components. The cost of the required equipment is also increasing dramatically. Lithography does not look like an option for true nanoscale construction.

B1.2 Scanning Probe Microscope-Aided construction: One approach to assembling nanoscale objects is to use an instrument that can move at these size scales, such as a Scanning Probe Microscope (SPM). Serious obstacles to using SPM to construct practical devices include the sequential nature of the technology and its controllability and scalability. The approach uses the tip of an SPM to move a chosen MBB or atom to the desired position on the work-piece being constructed. However, although substantial progress has been made, there are still serious obstacles, which may be insurmountable, to using SPM to construct practical devices. A key problem is that the detailed structure of the SPM tips is not well characterized, and furthermore does not have any gripping activity, that would allow controllable modulation of the affinity of a tip towards the MBB (or atom) that is manipulated. Therefore it is still very difficult to selectively pick up and transport a molecular object. Additionally, an SPM-assembly process constructs only one complex device at a time, and does not scale up if a substantial quantity of a device would be needed for real world applications.

B1.3 "Reprogramming" Biological Cells: Biological cells do operate on the nanoscale, but the machinery available in biological cells is exceptionally difficult to predict and control, and we are only beginning to full understand the complexity of its control systems.

B1.4 Supramolecular Chemistry and Protein Engineering: Because of the potential for greater control over fine molecular structures, this area holds great promise for molecular devices. However, it is moving slowly and does not share the programmable nature of biochemistry, such as DNA. Also, this approach when allied to proteins is at this time limited by the degree of predictability of the resulting protein conformations.

B1.5 Prior Chemical and DNA Self-assembly Methods: While self-assembly methods are well known and have been long used by chemists, they conventionally result in structures with limited complexity, and are not readily programmable. A very wide range of nanocrystals can now be produced, with a high degree of size and shape control [PMY+00, MSA+00, PKA01]. These inorganic nanocrystals may be metals, semiconductors, magnets, ferroelectrics, insulators, in short all the individual components of electrical devices. However, it remains difficult to arrange these nanocrystals into complex patterns as would be needed for making complete electrical devices. Recently, methods that use biopolymers to assemble nanocrystals have appeared in the literature. DNA is an ideal template for the formation of nanocrystal arrangements due to its ability to form well-defined and programmable secondary and tertiary structures and its similarity in size to nanocrystals. DNA has been used previously in the programmed assembly of particles. For example, (i) Mirkin *et al.* [MLM+96,MML99,M00] and Alivisatos *et al.* [LCP+99,AJP+96] have successfully attached oligonucleotide-derivatized nanoparticles to DNA using hybridization techniques, (ii) Mirkin [MLM+96] and Letsinger, [MSM+98] have shown that Au nanocrystals derivatized with complementary single-stranded DNAs (ssDNAs) can be hybridized to each other to form periodic lattices, (iii) Alivisatos[M00] bound gold particles to both single-stranded and double-stranded DNA modified with thiol groups, and (iv) Alivisatos and Schultz and coworkers [PWA+97] showed that nanocrystals modified with ssDNA could be arranged into dimeric and trimeric assemblies. (v) Niemeyer and coworkers conjugated streptavidin to single-stranded DNA oligonucleotides, hybridized the conjugates to a complementary RNA template, and bound biotinylated gold clusters to the streptavidin [NBP98], (vi) Cassell *et al.* assembled fullerene derivatives along the phosphate groups of the DNA backbone using cation exchange [CST98] and (vii) Coffey and coworkers formed rings of cadmium sulfide nanoparticles using double-stranded circular plasmid DNA attached to a solid substrate [CBP96].

B2 Other Methods for Crystallization. Other methods of crystallization exist for biological macromolecules. The standard methods have been treated extensively by McPherson [McP82] and by Ducruix and Giegé [DG92]. The methods that they discuss are basically trial and error methods. Carter & Carter [CC79] attempted to systematize the search for conditions using incomplete factorial methods [CC79], with limited success. Trial and error combined with the standard lore and kits available from such firms as Hampton Research (Laguna Niguel, CA) works about half the time. The other half the time one doesn't get crystals. If the hosts and guests can be ordered adequately, the system has several advantages:

- [1] Guests can be interchanged, so only one crystallization condition needs to be established.
- [2] Crystal contacts are not made by the object of study, so the crystals are likely to be much more robust in their responses to ligands, such as substrates, inhibitors and drugs.
- [3] One of the limiting factors in getting crystals from biological samples, micro-heterogeneity, is less likely to affect this system, because minor differences won't affect the quality of the lattice.

A key aspect of our system using 3D DNA lattices is that it is capable of fulfilling in 3D the original Schrödinger [Sch44] prescription for a living system in 1D, that it be 'an aperiodic crystal'. Thus, in addition to the periodic crystalline lattices that can form in 2D and 3D, it is also possible to incorporate motifs with different sequences, properties and sticky ends within the same framework. This is another unique feature of this system.

B3 Other Methods for DNA Effectors and Motors. It is worth noting that both the B-Z device and the PX/JX₂ device of Seeman are robust in both terminal states. The only other DNA-based device reported (Yurke's DNA tweezer [YTM+00]) has one robust state, and a second state that dimerizes. We find that the intermediate between our PX and JX₂ states, which corresponds to the dimerization state of [YTM+00], is ill-defined, but this has no impact on the operation of the device. We also explored other designs for DNA Effectors and Motors that combine ideas used in both these devices.

B4 Other Use of Nanocrystal/DNA Conjugates. The use of nanocrystal/DNA conjugates is being explored heavily by Mirkin and Letsinger. The Northwestern group is studying periodic lattices formed by nanocrystals with many oligonucleotides attached, as well as the use of scanning probe techniques for patterning in 2D. Christine Keating, Thomas Mallouk, and Theresa Mayer at Pen State are working on patterning of micron size metal barcodes using DNA. Both of these efforts are complimentary but do not overlap directly with our activity.